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DETOUR TRANSITIONS IN THE INTERNAL BREMSSTRAHLUNG SPECTRUM OF ^{91}Y

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ABSTRACT

The IB spectrum from ^{91}Y is measured in the energy region from 100–1250 keV by using a multichannel NaI (Tl) scintillation spectrometer with a standard geometrical arrangement. After effecting all the necessary corrections, the spectral shape, the total yields of intensity and of energy of IB are determined and compared with the corresponding theoretical (detour and direct) values of Ford and Martin in the energy region investigated. The present experimental results are in excess over the direct theory in the entire energy region investigated while they almost coincide with the detour theory in the energy region from 200 to 600 keV. Again below 200 keV and above 650 keV, the experimental results are deviating positively from even this detour theory. Above 650 keV the divergence between experiment and theory is found to increase with increasing energy.

I. INTRODUCTION

THE survey of Persson[1] in the field of IB reveals that there is a lack of agreement between theory and experiment in the majority of cases so far studied. The situation seems to be still worse for the case of forbidden beta decaying isotopes; always the experiment is exceeding the theory, the excess being higher the greater the photon energy. Several theories[2-10] of IB were developed and all of these earlier theories were found to be inadequate to explain the observed large experimental IB excess over theory. Of late, we find in the literature, attempts being made to explain the observed experimental IB excess over theory in terms of the so-called detour transitions[11-16]. Detour transitions are those in which the parent nucleus undergoes a virtual beta transition to a level of higher energy in the daughter nucleus which then decays to the ground state with emission of gamma radiation or *vice versa*. In these detour transitions the nucleus rather than the electrons radiate the photons unlike in direct transitions in which the photon is emitted by the outgoing (beta particle) electrons. Since the energy is not conserved in the intermediate state, the gamma spectrum is continuous. Ford and Martin[14] developed in detail, the theory of IB, taking into account the so-called detour transitions for the case of unique first forbidden beta decay.

To find out the importance of the detour transitions which are expected to be significant in forbidden beta decays, the investigation of IB from ^{91}Y was undertaken, since in this particular case, the earlier measurements[17-20] indicated a large discrepancy between theory and experiment. Further, in this case no attempts have so far been made to calculate in detail the detour effect and ascertain whether or not, it can account for the observed experimental excess over KUB theory.

II. EXPERIMENTAL DETAILS

The isotope ^{91}Y was obtained from the Bhabha Atomic Research Centre, Trombay, India, as carrier free solution with the least possible amounts of solids in it. In this isotope, it was found that there is a weak (0·3%) beta transition of energy 0·33 MeV which is followed by a gamma ray of energy of 1·2 MeV. Except for this gamma line, the source was found to be free from any other monoenergetic gamma rays or beta impurities when tested with a Ge (Li) detector (having a FWHM of 3·7 keV for 662 keV gamma rays from ^{137}Ba) and anthracene crystal.

The preparation of IB sources, the measurement, the method of analysis and the evaluation of various errors in the measurement as well as in the calculation of various correction factors were the same as those found in our earlier publication[20] except for the following improvements and differences.

To eliminate the air disturbances in the low energy region, the column between the detector and the source was evacuated and maintained at a pressure of 1 mm of mercury. Secondly, the single channel analyser employed in the earlier study was replaced by a 512 channel analyser of ND 1100 system along with a non-overloading amplifier of type PA 563 which facilitated collection of larger counts resulting in better statistical accuracy.

The overall error in the present investigation was estimated as the r.m.s. value of the different possible errors in the measurement of IB as well as in the calculation of various correction factors. The total r.m.s. error thus arrived at is of the order of 7% at 1000 keV and also it was found that this error decreases with decreasing energy being 6·08% at 700 keV and 2·74% at 200 keV.

III. Results and Conclusions

The theoretical IB distributions (as probability for bremsstrahlung per mc^2 energy interval per beta disintegration) for both the direct and detour transitions were calculated using the theory of Ford and Martin[14]. After effecting all the necessary corrections the final experimental distributions were all obtained as number of photons per mc^2 energy interval per beta disintegration.

^{91}Y is an unique first forbidden beta decaying isotope having an end point energy of 1545 keV and a half-life of 58·5 days. In this case, the comparison between the present experimental results of IB and the corresponding theoretical distributions in the region from 100 keV to 1250 keV is shown in Fig. 1. From this figure it is seen that the present experimental results are almost coinciding with the detour corrected theory due to Ford and Martin[14] from 200 to 650 keV, the experiment is deviating positively from even the detour theory. The experimental excess over detour theory at 800 keV is 75% and at 900 keV is 120%.

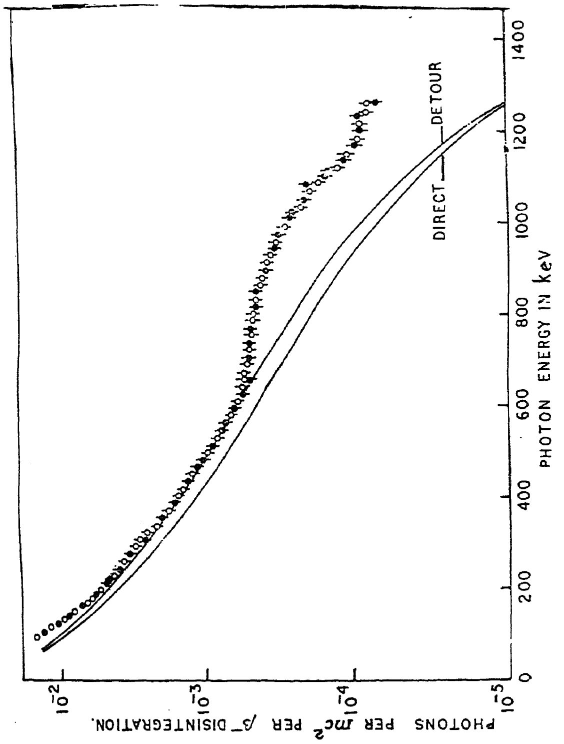

Fig. 1. ^{91}Y Internal bremsstrahlung Intensity Spectrum, ○ Experimental data with different source strengths ● ranging from 50 to 150 micro Curies.

Abscissa—Photon energy in keV.

Ordinate—Photons per mc^2 per beta disintegration.

The IB from this isotope was hardly studied previously. Even in the relatively few earlier investigations available for this case, the contribution of detour transitions to IB was not considered. In the first measurement due to Bolgiano *et al*[19] which was confined only to a very small energy region from 20 to 150 keV, there was good agreement between experiment and theory (KUB). Subsequently, two more measurements[17,20] were made of IB from the same isotope which covered the high energy as well, upto 1000 keV. These two later studies, namely, the differential measurements of IB due to Persson[17] at large angles between the emitted electrons and the accompanying IB photon as well as the integral (single counter) measurements of Narasimha Murty *et al.*[20] represent similar trends of the experimental results of IB with respect to Coulomb corrected KUB theory, in the sense that in both of them, there was experimental excess over theory throughout the investigated energy region from 100 to 1000 keV, the experimental excess over theory increasing with increasing photon energy.

When the present results are compared with those of Persson and of Narasimha Murty *et al.*, it is seen that the present experimental values are also having similar behaviour in respect of deviating from the direct theory throughout the investigated energy region. But, the extra finding that can be claimed in the present study is the successful applicability of the detour theory in the photon energy region from 100 to 650 keV as can be seen from Fig. 1.

An attempt to improve the agreement between the theory and experiment even at high energies, by assuming the amplitudes of detour matrix elements to be twice or thrice as large as the direct matrix elements instead of equal magnitude, was also made in the present investigation and found to be unsuccessful since such an assumption resulted in increasing the detour contribution to a greater extent, once again, only at the middle energy region.

In Fig. 1., the small vertical lines over each experimental point are a measure of the total r.m.s. value of the error involved in the experimental measurements at that energy. For the experimental points for which no limits of error are given, it is implied that the limits lie within the symbols. The total yields of intensity and of energy of IB from this isotope in the investigated energy region of 100 to 1250 keV is determined to be $3·809 \times 10^{-3}$ ($2·569 \times 10^{-3}$; $2·994 \times 10^{-3}$) and $2·146 \times 10^{-3}$ ($1·395 \times 10^{-3}$; $1·741 \times 10^{-3}$) respectively. The values in the brackets represent the corresponding quantities for direct and detour theories respectively for the same energy region.

Finally, it is concluded that throughout the investigated energy region, the experimental results are deviating positively from the direct theory and this positive deviation is found to be increasing with increasing energy. But the detour theory is found to be successful in explaining the experimental excess over the theory only in the middle energy region. It is therefore desirable to develop new theories by taking into account new effects if there are any, which were ignored in the existing theories and which are likely to augment the IB intensities especially at high photon energies.

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1. Persson, B., *Pre. Conf. on the Electron Capture and Higher Order Processes in Nuclear Decays,* Debreceen, 1968.
2. Knipp, J. K. and Uhlenbeck, G. E., *Physica,* 1936, 3, 425.
3. Bloch, F., *Phy. Rev.*, 1936, **50**, 272.
4. Chang, C. S. W. and Falkoff, D. L., *Ibid.*, 1949, 56, 365.
5. Madansky, L., Lipps, F., Bolgiano, P. and Berlin, T. H., *Ibid.,* 1951, **84**, 596.
6. Nilsson, S. B., *Ark. Fys.*, 1956, **10**, 467.
7. Lewis, R. R. and Ford, G., *Phy. Rev.*, 1957, 107, 756.
8. Vinh-Mau, R., *Nuovo Cimento,* 1961, **19**, 609.
9. Spruch, L. and Gold, W., *Phy. Rev.*, 1959, 113, 1060.
10. Felsner, G., *Z. PHY.*, 1963, **174**, 43.
11. Longmire, C., *Phy. Rev.*, 1949, **75**, 15.
12. Horowitz, J., *J. PHY. et Radium,* 1952, **13**, 429.
13. Hakeem, M. A. and Goodrich, M., *Nucl. Phy.*, 1962, 31, 322.
14. Ford, G. W. and Martin, C. F., *Ibid.*, 1969, 134 A, 457.
15. Beattie, R. J. D. and Byrne, J., *Ibid.,* 1971, 161 A, 650.
16. Powar, M. S. and Singh, M., *J. Phy.*, 1972, 5, 460.
17. Persson, B., *Nucl. Phy.*, 1964, **55**, 49.
18. —, *Ibid.*, 1965, **67**, 121.
19. Bolgiano, P., Madansky, L. and Rasetti, F., *Phy. Rev.*, 1953, **89**, 679.
20. Narasimha Murty, K. and Jnanananda, S., *Proc. Phy. Soc.,* 1967, p. 109.

EXPLOSIVE PHASE TRANSFORMATION IN $Sm_{0.8}$ $Tb_{0.2}$ S

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We recently reported the thermo-power study of the isostructural electronic phase transition in SmS which occurs around 6·5 Kbar pressure[1]. The semiconductor to metal transition manifests itself as a dramatic drop in thermo-power (Q) followed by a change of sign. After the phase transition, Q increases anomalously over a narrow pressure range. It is well known that the high pressure phase of SmS is in a mixed valance or interconfigurational fluctuation state[2] (ICF). Further the effect of pressure can be simulated by substituting smaller trivalent ions like Tb^{3+}, Gd^{3+}, Y^{3+}, etc., for the larger divalent Sm ions in the SmS lattice[3,4]. Thus the critically doped and 'Chemically collapsed' systems like $Sm_{0.8}$ $Tb_{0.2}$ S, $Sm_{0.84}$ $Gd_{0.16}$ S, etc., exhibit properties which are similar to the high pressure phase (metallic phase) of SmS.

In this communication we report some new data on the thermoelectric behaviour of the chemically collapsed $Sm_{0.8}Tb_{0.2}S$ system in the temperature range $-200°$ C to $+200°$ C. Further this study gives evidence that the black phase obtained after the explosive phase transformation[3,5] at $-170°$ C (B′-Phase) is quite distinct from the normal black phase (B-phase).

Figure 1 gives the temperature-concentration diagram[3] of a typically doped material like Sm_{1-x} Gd_xS. For concentrations less than the critical value the material will be in the B–Phase (FCC) which is the normal semiconducting phase. For concentrations greater than a critical value and for temperatures above 100° K, the black phase undergoes an isostructural first order phase transformation to a metallic phase (M). This metallic phase on cooling undergoes an explosive phase transformation to the B′–phase, near liquid nitrogen temperature. There is evidence to indicate that this is also an isostructural transformation[3]. As mentioned earlier this study clearly shows that B and B′ are electronically distinct phases.

Figure 2 presents the variation of Q with temperature ranging from $-200°$ C to $+200°$ C for $Sm_{0.8}Tb_{0.2}$S. The most notable feature is the change of sign of Q at lower temperatures followed by very large increase in its magnitude ($Q \approx -40\ \mu v/°$ C at $-150°$ C). The explosive phase transformation from the metallic to the B′-phase is seen by the large discontinuity in Q occurring near $-170°$ C. The sign of Q is positive in the B′–phase. From our earlier studies[1], we note that Q is negative in the B–phase

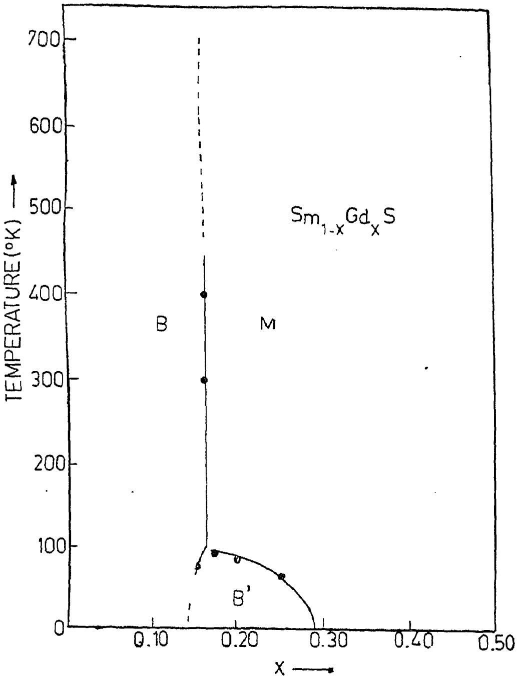

FIG. 1. Phase diagram of $Sm_{1-x}Gd_xS$ system. B—Normal semiconducting phase; M—Metallic phase; B′—New black phase after the explosive phase transformation.

which suggests that the electronic structures of the B and B′ phases are different. The decrease of Q at higher tempetatures leading to a change of sign around + 200° C is associated with the continuous phase transformation from the metallic to the B–phase.

Figure 3 gives the pressure dependence of Q in $Sm_{0.8}Tb_{0.2}S$ and in the metallic phase of SmS. It may be noted that over a narrow pressure range, the thermo-power increases anomalously in both these systems and their remarkably identical behaviour is indicative of the close similarity in their electronic structure.

The anomalous temperature and pressure dependence of Q in these 4*f* substances can be qualitatively understood on the basis of the Hirst Model[2] according to which the 4*f* band with a high density of states lies at the Fermi level. The central result of Hirst's model is that the Samarium ions in the high pressure phase of SmS or in the chemically collapsed $Sm_{0.8}Tb_{0.2}S$ are rapidly undergoing inter-configurational fluctuations between the iso energetic $4f^65d^0$ and $4f^55d'$ states. For temperatures $kT << \Delta$, where Δ represents the width of the 4*f* resonance, the ions would be in a well defined configuration and when $kT >> \Delta$, the ionic configuration will be rapidly varying leading to the

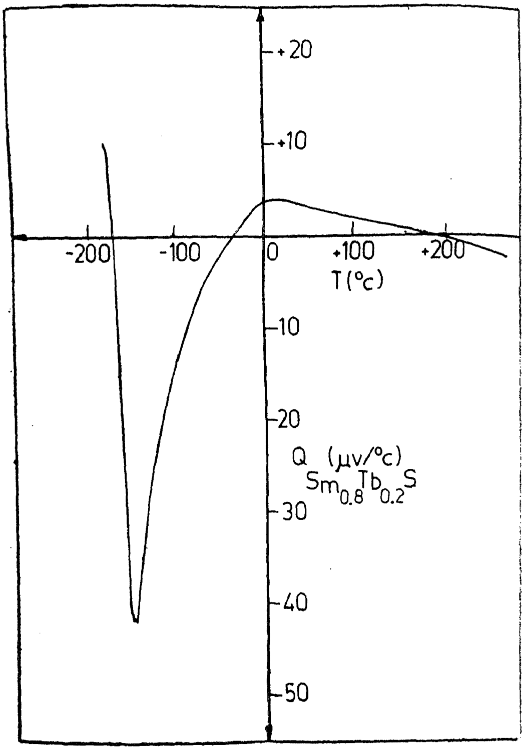

FIG. 2. Explosive phase transition in Sm $Tb_{0.2}$S. The discontinuity in Q near −170° C corresponds to the M–B′ phase transformation.

phenomenon of 'mixed valence'. Also the application of pressure will move the Fermi level away from the centre of 4*f* resonance which again will lead to a continuous change in the fractional valence. The amount of fractional valence can thus be varied continuously by the application of pressure or temperature.

It is well known that the thermo-power of a metal depends on the energy derivative of the relaxation time (τ) and the density of states at the Fermi level. The anomalous increase of thermopower with decrease of temperature (Fig. 2) suggests that $[d\tau/dE]_{E_F}$ is positive, (which makes a negative contribution to thermopower) and its magnitude increases with decrease of temperature. Since the concentration of electrons possessing 5*d* character, which effectively carry the current decreases with decrease of temperature the magnitude of Q would become large at lower temperatures. The decrease in Q at higher temperatures (0° C to 200° C) can be correlated with the continuous change of valence of Samarium ions towards the divalent state.

The anomalous pressure behaviour (Fig. 3) can be explained if one assumes that with the increase of pressure the Fermi level probes the region of density of states lying below the centre of 4*f* resonance. This gives rise to $[d\tau/dE]_{E_F}$ becoming negative with pressure leading to large positive values of Q. It is of interest to study the low temperature behaviour of the high pressure phase of SmS and experiments in this direction are in progress in our laboratory.

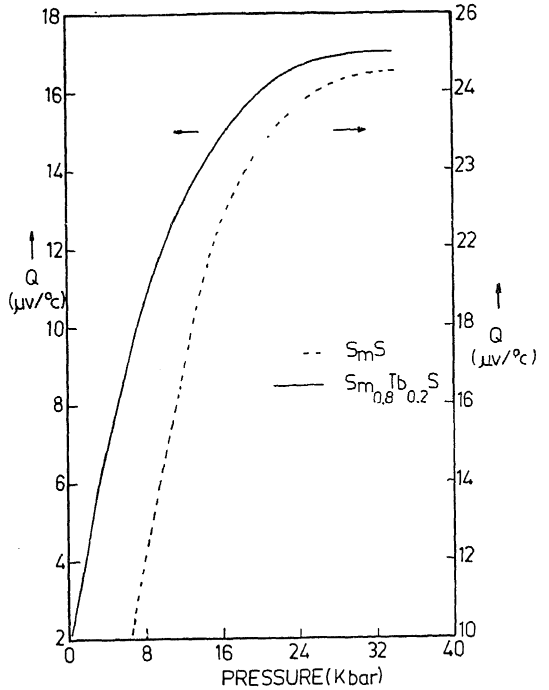

FIG. 3. Q *versus* P graph in $Sm_{0\cdot 8}Tb_{0\cdot 2}S$ and in the high pressure phase of SmS.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. A. Jayaraman, for providing us with the samples and to Dr. S. Ramaseshan, for his keen interest in this work. One of us (V. Shubha), would like to acknowledge C.S.I.R., for the grant of a Fellowship.

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1. Ramesh, T. G. and Shubha, V., *Solid State Comm.*, 1976, 19, 591.
2. Hirst, L. L., *Phys. Kond. Mat.*, 1970, 11, 255.
3. Jayaraman, A., Dernier, P. D. and Longinotti, L. D., *Phys. Rev.*, 1975, 11 B, 2783 (and references therein).
4. Tao, L. J. and Holtzberg, F., *Ibid.*, 1975, 11 B, 3842.
5. Ramesh, T. G. and Shubha, V., *Proceedings of the International Conference on Valence Instabilities and Related Narrow Band Phenomenon Held in Rochester*, New York, 1976.

HIGH ENERGY SINGLE AND DOUBLE PLASMON SATELLITES IN THE Be AUGER SPECTRUM

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ABSTRACT

Relative intensities of high energy single and double plasmon satellites in the Be Auger spectrum have been calculated as 0·0058 and 0·0061 respectively. The calculated values agree fairly well with the corresponding experimental values 0·0053 and 0·0047 of Jenkins *et al.*

SHMIDT[1], Steinmann[2] and Neddermeyer and Wiech[3] have shown theoretically that the involvement of plasmon in the emission of high energy satellites is possible. Evidence for the existence of high energy plasmon satellities have been shown by several workers[4-9]. Recently Jenkins *et. al.*[5] have observed two high energy satellites at energy distances of 18 eV and 38 eV from the main Be (KVV) Auger peak on the high energy side. They have assigned the first satellite $(KVV + \hbar w_p)$ as due to single plasmon energy gain by the Auger electron, while the second satellite at 38 eV has been assigned $[(K)_2\ VV]$ as due to double ionization. The last assignment is based on the rough approximation which Jenkins and Zehner[5] have made for the ionization energy of the second K-electron of Be. Recent calculations by Hayasi[10], assuming double ionization, place the main band-high energy satellite separation at 34·07 eV for Be. This value does not agree with the experimental value of either Jenkins *et. al.*[5] (38 eV) or of Hayasi[11] (37·6 eV). In fact the experimental values of both the workers are in excellent agreement with the double plasmon energy $(2\ \hbar w_p) = (38\text{ eV})$. Thus on the basis of energy consideration the high energy satellite at 38 eV can be assigned as due to double plasmon energy gain, rather than the double ionization process.

Jenkins and Zehner[5] have also observed two low energy satellites at 18 eV and 38 eV to which they claim respectively as due to single and double plasmon creation by the Auger electrons. That is, the existence of plasmons up to several orders in Be is possible[12]. It is also possible that on decay, plasmons can give their energy to Auger electrons giving rise to high energy plasmon satellities up to several orders. Thus the satellites at 18 eV and 38 eV on the high energy side of main Auger peaks may be respectively due to single and double plasmon energy gain process. The first satellite at 18 eV has already been assigned by Jenkins and Zehner[5] as due to single plasmon energy gain process. The second satellite at 38 eV, on the basis of energy consideration can be assumed as due to plasmon energy gain process.

A further check to the double plasmon energy gain process is the calculation of the relative intensity of this satellite with respect to the main peak. Following Langreth[13-14], the relative intensity of the double plasmon satellite can be given as:

$$i = \frac{\cdot 06\,\gamma^2}{2\,e^{-\gamma}} \tag{1}$$

where

$$\gamma = \frac{\gamma_s}{6} \tag{2}$$

γ_s is a dimensionless parameter and is given[15] by:

$$\gamma_s = \left(\frac{47\cdot 11}{\hbar\,\omega_p}\right)^{2/3} \approx 1\cdot 9 \tag{3}$$

From equation (1) the calculated value for i is 0·0061 which is in close agreement with the experimental value 0·0047 of Jenkins[16] (*see* Table I).

TABLE I

Relative intensity of single and double plasmon satellite in Be Auger spectrum

Metal	α	β	$\hbar w_p$ (eV)	γ_s	Relative intensity (i)			
					Authors' value		xperimental value	
					Single plasmon	Double plasmon	Single plasmon	Double plasmon
Be	0·3577	0·71	18·0	1·9	0·0058	0·0061	0·0053	0·0047

Thus the satellite at 38 eV on the high energy side of the main Auger peak KVV may be due to double plasmon energy gain process rather than double ionization process and thus can safely be given the following assignment (KVV + $2\hbar w_p$).

The relative intensity of the first plasmon satellite at 18 eV ($\hbar w_p$) can also be calculated using Srivastava *et. al.*[7-9] and Brouers[17] formula:

$$i = \alpha\left[1 - \frac{3}{2}\sqrt{\frac{2}{\beta}}\tan^{-1}\sqrt{\frac{\beta}{2}} + \frac{1}{2}\,\frac{1}{(1+\beta/2)}\right] \tag{4}$$

where $\alpha = 0\cdot 3577$, $\beta = 0\cdot 71$ (See reference 15).

The calculated value of i from equation (4) 0·0058 which is in fair agreement with the expe mental value (0·0053) of Jenkins[16] (*see* Table I

The relative intensities of both satellites, *i.e.*, energy distances of $\hbar w_p$ and $2\hbar w_p$ (0·0058 0·0061) which are approximately of the same or of magnitude, satisfy the general characteristic the plasmon involvement. This again strengthe our argument. Thus we can safely say that h the satellites on the high energy side of the m Auger peak of Be spectrum are due to plasm energy gain process and they can be given respe tively the following assignment (KVV + $\hbar w_p$) a (KVV + $2\hbar w_p$).

ACKNOWLEDGEMENT

Thanks are due to Dr. S. C. Gupta, Principal the College, for encouragement, Mr. V. Kum for checking the calculations and to U.P. Sta C.S.T., for financial assistance.

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1. Shmidt, V. V., *Soviet Phys. JETP.*, 1961, 1 886.
2. Stainmann, W., *Phys. Stat. Sol.*, 1968, 28, 43
3. Neddermeyer, H. and Wiech, G., *Phys. Le* 1970, **30 A**, 17.
4. Hanson, W. F. and Arakawa, E. T., *Z. Phys* 1972, 251, 271.
5. Jenkins, L. H. and Zehner, D. M., *Solid Sta Commun.*, 1973, 12, 1149.
6. Arakawa, E. T. and William, M. W., *Phy Rev.*, 1975, 8 B, 4075.
7. Srivastava, K. S., Singh, S. P. and Shrivastava R. L., *Phys. Stat. Sol.* (in press),
8. —, — and —, *Phys. Lett.*, 1974, **47 A**, 305.
9. —, Shrivastava, R. L. and Singh, S. P., *Phys. Stat. Sol.* (in press).
10. Hayasi, Y., *Sci. Rep. Tohoku Univ.*, 1969, 52 (1), 41.
11. —, *Ibid.*, 1968, 51 (1) 1.
12. Jenkins, L. H., Zehner, D. M. and Chung M. F., *Surface Sci.*, 1973, **38 A**, 327.
13. Langreth, D. C., "Collective properties o Physical system," *Nobel*, 1973, 24, 210.
14. —, *Phys. Rev. Lett.*, 1971, 26, 1229.
15. Rook, G. A., *Soft X-ray Band Spectra*, Edite by D. J. Fabien, Academic Press, 1968, p. 5
16. Jenkins, L. H., Private Communication.
17. Brouers, F., *Phys. Lett.*, 1964, 11, 297.

MAGNETIC SUSCEPTIBILITY OF COBALTIC COMPLEXES

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ABSTRACT

Cobaltic complexes of the type $[Coen_2(R)Cl]Cl_2$ where 'R' is an aliphatic or aromatic amine were prepared in a chemically pure state and their magnetic susceptibilities were measured by Guoy method. All complexes were found to be diamagnetic. The susceptibility values were compared with their experimentally computed values assuming strict additivity. The deviations from additivity have been explained on the basis of Van-Vleck's equation.

EXPERIMENTAL

COBALTIC complexes of the type $[Coen_2(R)Cl]Cl_2$ where 'R' is an aliphatic or aromatic amine were prepared in a pure state by following the method suggested by john C. Bailer Jr. and L. B. Clapp[1]. The starting material namely trans-di-chloro-diethylenediamine-cobaltic chloride was also prepared in the laboratory as described by Furnelius[2]. Cobalt chloride was of B.D.H. 'Analar' grade. The organic bases were of A.R. quality and they were further purified by conventional methods. Their purity was checked by their physical constants.

The purity of the complexes was ascertained by analysing them for nitrogen and chloride contents. Magnetic susceptibilities of these complexes and their components were measured on a modified form of Guoy[3] balance described by Prasad and co-workers[4]. All the complexes were found to be diamagnetic. The results of these measurements are given in Table I in which χ and χ_m denote respectively the specific and molar susceptibilities expressed in -1×10^{-6} c.g.s. units.

Assuming strict additivity the molar magnetic susceptibilities of these complexes were computed

TABLE I

Sr. No.	Complexing ligand	Formula of the complex	Colour of the complex (Time of reaction)	Elemental Analysis: Nitrogen Calc. (Found)	Elemental Analysis: Chloride Calc. (Found)	Molecular weight of the complex	χ	χ_m	$\Sigma\chi_d$ (Computed)	χ_p = $\Sigma\chi_d - \chi_m$
1.	Dicyandiamide	$[Coen_2(C_2H_4N_4)Cl]Cl_2$	Bright-red (2 hr)	—	28·81 (29·05)	369·72	0·234	86·32	218·05	131·73
2.	Pyridine	$[Coen_2(C_6H_5N)Cl]Cl_2H_2O$	Bright crimson red (0·5 hr)	—	27·84 (27·31)	382·74	0·184	70·50	223·74	153·24
3.	Ortho-phenetidine	$[Coen_2(C_6H_4NH_2OC_2H_5)Cl]Cl_2$	Pale-red (24 hr)	16·57 (15·90)	25·21 (24·80)	422·40	0·269	113·4	267·10	153·70
4.	Meta-toluidine	$[Coen_2(C_6H_4NH_2{-}C_3)CHl]Cl_2H_2O$	Pale-crimson (0·5 hr)	17·05 (16·70)	27·14 (27·50)	392·40	0·212	82·99	249·13	166·14
5.	Meta-chloro-aniline	$[Coen_2(C_6H_4NH_2{-}Cl)Cl]Cl_2$	Crimson red (24 hr)	16·80 (15·90)	34·37 (34·43)	413·21	0·196	81·00	252·12	171·12
6.	Paraanisidine	$[Coen_2(C_6H_4NH_2{-}OCH_3)Cl]Cl_2.H_2O$	Dirty pink (0·5 hr)	16·41 (15·90)	24·94 (24·30)	426·40	0·161	68·77	255·17	186·40
7.	Ortho-chloro-aniline	$[Coen_2(C_6H_4.NH_2.Cl)Cl]Cl_2$	Purple (24 hr)	16·80 (16·50)	34·37 (34·50)	413·21	0·108	44·63	253·02	208·39
8.	Meta-anisidine	$[Coen_2(C_6H_4.N_2H{-}OCH_3)Cl]Cl_2$	Pink red (0·25 hr)	16·41 (15·80)	24·94 (24·85)	426·40	0·108	46·03	254·56	208·53
9.	Meta-Phenetidine	$[Coen_2(C_6H_4.NH_2{-}OC_2H_5)Cl]Cl_2$	Pink red (24 hr)	16·57 (16·00)	25·21 (25·50)	422·40	0·130	54·94	266·42	211·48
10.	Benzidine dine	$[Coen_2(C_6H_5NH_2{-}C_6H_5NH_2)Cl]Cl_2$	Dirty vilolet (0·25 hr)	11·92 (12·00)	22·67 (21·91)	469·87	0·160	75·21	290·12	214·91

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*Molecular weights of the complex were determined ebullioscopically.

$(\Sigma \chi_d)$ by adding experimentally observed values of the susceptibilities of their components. The deviations in the observed and the computed values are shown in the last column of the same table.

DISCUSSION OF THE RESULTS

It is evident from the results that 'the molar magnetic susceptibilities of the complexes are all dimagnetic. It is, therefore, concluded that all of them are spin paired complexes of trivalent cobalt. Further it is seen that the observed values of molar susceptibilities of these complexes are much lower in magnitude than their computed values. Guoy method gives results up to 1% accuracy. These deviations are therefore outside the limits of experimental error and are, therefore, significant.

According to Van-Vleck[5], the molar susceptibility of a polyatomic molecule in Σ state may be written as $\chi_m = \chi_d + \chi_p$, where χ_d represents the dimagnetic term which is a function of all the electronic orbits in the molecule and χ_p represents second order paramagnetic term independent of temperature which arises on account of mixing of the ground and excited states of electrons in the molecule. Since the nature of the various ligands complexing with the cobalt ion do not differ much, the contribution of χ_d term to the χ_m value of the complex is not likely to be affected considerably. Therefore it may be concluded that there is a substantial contribution of the temperature independent paramagnetism due to cobalt atom in all these complexes. The difference $[(\Sigma \chi_d - \chi_m) = \chi_p]$ thus gives the residual paramagnetism. The χ_p values thus calculated are given in the last column of Table I. Since all the complexes studied in this investigation are of the type $[\mathrm{Coen_2(R)Cl}]\mathrm{Cl_2}$, where 'R' is an aliphatic or aromatic amine, comparison of these χ_p values would be possible. Examination of these results show that these values vary with the nature of the ligand 'R'. The data indicate that χ_p values decrease in the following order of 'R':

Benzedine > *m*-phenetidine > *m*-anisidine > *o*-chloroaniline > *p*-anisidine > *m*-chloroaniline > *m*-toluidine > *o*-phenedidine > pyridine > dicyandiamide.

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1. John, C. Bailer Jr. and Clapp, L. B., *J. Am. Chem. Soc.*, 67, p. 171.
2. Furnelius, *Inorganic Synthesis*, Vol. II.
3. Guoy, *Compt. Rend.*, 1889, 109, 936.
4. Prasad and co-workers, *Proc. Ind. Acad. Sci.* 1944, 20 A, 224.
5. Van-Vleck., *Theory of Electric and Magnetic Susceptibilities*, Oxford University Press, 1932.

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LETTERS TO THE EDITOR

PREPARATION AND PRELIMINARY X-RAY STUDIES OF ARGININE ASCORBATE, A CRYSTALLINE COMPLEX BETWEEN AN AMINO ACID AND A VITAMIN

ENZYME-COENZYME interactions, which are mostly noncovalent in character, are of considerable importance in biology. One approach to the study of the atomic details of such interactions is through the x-ray analysis of crystalline complexes between amino acids and short peptides on the one hand, and coenzymes and vitamins on the other. Here we report the preparation and preliminary x-ray analysis of such a complex between L-agrinine and L-ascorbic acid (Vitamin C). Ascorbic acid is also known to function as a coenzyme in some enzymatic reactions. It may be mentioned that the work reported in this note constitutes the first attempt at the x-ray analysis of a crystalline complex between an amino acid and a vitamin or a coenzyme.

The crystals of L-arginine L-ascorbate were prepared by the slow evaporation of an aqueous solution of the components in molar proportions. In order to prevent the oxidation of ascorbic acid, the crystallization experiments were conducted inside a desiccator in nitrogen atmosphere. An alkaline solution of pyrogallic acid was placed in the desiccator to remove the residual oxygen. The composition of the crystals was confirmed by comparing the ultraviolet absorption spectrum of the crystals with those of the components.

The space group and the unit cell dimensions of the crystals were determined from oscillation and Weissenberg photographs taken about crystallographic axes using $CuK\alpha$ radiation. The density of the sample was measured by flotation in a mixture of benzene and carbon tetrachloride. These data are given below.

Space group $P2_1$

$a = 5\cdot060 \pm 0\cdot008$, $b = 9\cdot977 \pm 0\cdot009$,
$c = 15\cdot330 \pm 0\cdot013$ Å,
$\beta = 97\cdot5 \pm 0\cdot2°$, $D_m = 1\cdot509 \pm 0\cdot008$,
$D_c = 1\cdot516$ gm/cc, $Z = 2$.

The complete structure determination of the complex is in progress.

The authors thank Professor G. N. Ramachandran, for his kind interest in the work. Their thanks are also due to the University Grants Commission, for financial support and for the award of a Junior Research Fellowship to one of us (V.S.).

Molecular Biophysics Unit,
Indian Institute of Science,
Bangalore 560 012,
May 4, 1976.

V. SUDHAKAR.
M. VIJAYAN.

DETERMINATION OF PHENANTHRIDINE IN NON-AQUEOUS MEDIA

PHENANTHRIDINE assumes importance as its salts are found to be remarkably active against trypanosome infections[1]. Very few methods are available for the determination of the base. In the present work an attempt has been made to determine phenanthridine in ethyl methyl ketone-acetic acid medium using chlorosulphonic acid by employing visual, photometric and potentiometric techniques.

Chlorosulphonic acid was purified by fractional distillation. Ethyl methyl ketone and acetic acid were purified by standard methods[2,3], Phenanthridine was directly used after checking its melting point (108° C).

Approximately 1 M chlorosulphonic acid in acetic acid was diluted with ethyl methyl ketone to get the required concentrations. The solution was standardised by titration with anhydrous sodium acetate dissolved in the mixed solvent both potentiometrically and by visual titration.

In visual titrations 25 ml. of 0·02 M phenanthridine solution was tritrated with 0·05 M chlorosulphonic acid using methyl orange indicator. The indicator produced light yellow colour in base solutions and bright pink in acid solutions.

In photometric titrations, 25 ml. of 0·0001 M phenanthridine solution was tritrated against 0·01 M chlorosulphonic acid in presence of methyl red at 490 mμ.

In potentiometric titrations 25 ml. of 0·01 M base solution was titrated with 0·05 M chlorosulphonic acid. Glass electrode functioned as indicator electrode and the calomel electrode acted as reference electrode.

The experimental results obtained with the aid of the three different techniques are given in Table I.

TABLE I

	Phenanthridine		
	Taken, mg	Found, mg	Error (%)
Visual	17·92	17·96	+0·2
	8·96	8·92	−0·4
Photometric	0·89	0·89	0·0
	0·45	0·45	0·0
Potentiometric	8·96	8·96	0·0
	4·48	4·50	+0·4

The results show that chlorosulphonic acid could successfully be employed as a titrant for the determination of semi-micro quantities of phenanthridine.

Physico-Chemical Labs., K. C. MOHAN RAO.
S.V. University, P. R. NAIDU.
Tirupati (A.P.), *May* 13, 1976.

1. Acheson, R. M., *An Introduction to the Chemistry of Heterocyclic Compounds*, Interscience, New York, 1967, p. 285.
2. Vogel, A. I., *Practical Organic Chemistry*, Longmans, London, 1959.
3. Kucharsky, J. and Safarik, L., *Titrations in Non-Aqueous Solvents*, Elsevier, New York, 1965.

NICKEL (II) COMPLEXES OF SOME BIDENTATE AND TRIDENTATE SCHIFF BASES

THE chemistry of metal complexes of tridentate dibasic Schiff bases has received considerable attention in recent years[1-3]. Tridentate dibasic ligands force the M^{2+} ions to form dimeric or polymeric metal complexes resulting in compounds with novel magnetic and spectral properties. In continuation of our work on tridentate dibasic Schiff bases[4] we report in this communication the synthesis of several nickel (II) complexes of the Schiff bases (I) derived from salicylaldehyde, 2-hydroxy-1-naphthaldehyde and ethanolamine or propanolamine. The complexes have been characterised by elemental analysis, infrared and electronic spectra, conductance and magnetic susceptibility measurements.

The complexes were prepared by refluxing a mixture of nickel (II) acetate tetrahydrate (0·005 mole in 30 ml 95% ethanol) and the condensed Schiff base (0·005 mole in 20-40 ml ethanol 95%) on a steam bath for 2 hours. The separated compounds were filtered, washed with 95% ethanol and dried at 90° C for one hour. The complexes with N-(hydroxyalkyl) salicylideneimine were recrystallised from methanol or chloroform. The complexes with N-(hydroxyalkyl)-2-hydroxynaphthylideneimine are insoluble in common organic solvents. The complexes gave satisfactory elemental analysis.

The magnetic, infrared and electronic spectral data of the complexes are presented in Table I.

The complexes exhibit the ν (OH) stretch in the region 3100–3300 cm^{-1}. The observation of ν (OH) stretch indicates the presence of coordinated water in $[Ni(H_2O)_3L]$ complexes and of non-coordinated hydroxyl group of the coordinated ligands in $[NiL'_2]$. The ν (C=N) frequency of the complexes occurs in the range 1605–1630 cm^{-1}. The shift of the ν (C=N) frequency to lower wavenumber in comparison to the free ligands (1640 cm^{-1})[5] indicates the coordination of the Schiff bases through the nitrogen atom of the azomethine group. The magnetic moments (3·0 – 3·2 B.M.) of the hexa-coordinated complexes $[Ni(H_2O)_3L]$ as determined by the Guoy method support the presence of an octahedral structure[6]. The tetra-coordinated complexes $[NiL'_2]$ are diamagnetic and a square planner structure can be assigned to these complexes[6]. The $[Ni(H_2O)_3L]$ complexes exhibit two ligand field bands in methanol solution at about 10000 and

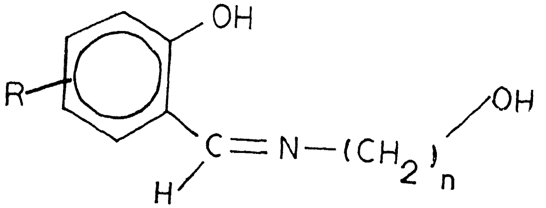
TABLE I

Magnetic, infrared and electronic spectral dala of nickel (II) schiff base complexes

Complex	μ_{eff} B.M.	$\nu_{(C=N)}$ Cm^{-1}	Medium	Absorption spectral data[a], cm^{-1}
[Ni (H$_2$O)$_3$ salicylaldehyde-ethanolamine)]	3·19	1630	methanol	16150 (8·1), 10500 (10·3)
[Ni (H$_2$O)$_3$ (salicylaldehyde-propanolamine)]	3·29	1610	methanol	15050 (10·7), 9750 (7·6)
[Ni (hydroxynaphthaldehyde-ethanolamine)$_2$]	diamagnetic	1605	reflectance	17400, 16200
[Ni (hydroxynaphthaldehyde-propanolamine)$_2$]	diamagnetic	1605	reflectance	17400, 16300

[a] Figures in the parenthesis indicate ϵ.

16000 cm^{-1} due to the spin-allowed transitions $^3A_{2g} \rightarrow {}^3T_{2g} \rightarrow$ and $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ respectively in an O_h symmetry[6]. The low molar absorptivity of theses bands (6–10 litre cm^2 mole^{-1}) is in accordance with an octahedral structure[7]. The reflectance spectra of [NiL$'_2$] complexes exhibit two bands at around 16300 and 17400 cm^{-1} and these bands are characteristic of square planar structure with NiN_2O_2 coordination sphere[7,8].



I. R = H, C_4H_4;
n = 2, 3.

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---

1. Hatfield, W. E. and Whyman, R., *Transition Metal Chemistry*, 1969, 5, 47.
2. Hodgson, D. J., *Progress Inorganic Chemistry*, 1975, 19, 1.
3. Syamal, A., *Coord. Chem. Rev.*, 1975, **16**, 309.
4. Lee, C. C., Syamal, A. and Theriot, L. J., *Inorg. Chem.*, 1971, 10, 1669; Syamal, A., *Indian J. Chem.*, 1973, 11, 363; Carlisle, G. O., Syamal, A., Ganguli, K. K. and Theriot, L. J., *J. Inorg. Nucl. Chem.*, 1972, 34, 2761; Syamal, A. and Theriot, L. J., *J. Coord. Chem.*, 1973, 2, 193, 241; Syamal, A., Carey, E. F. and Theriot, L. J., *Inorg. Chem.*, 1973, 12, 245; Syamal, A. and Kale, K. S., *Curr Sci.*, 1975, **44**, 256.
5. Daasch, L. W. and Hanninen, U. E., *J. Am. Chem. Soc.*, 1950, 72, 3673.
6. Cotton, F. A. and Wilkinson, G., *Advanced Inorganic Chemistry*, Wiley Eastern Pvt. Ltd., 2nd Edition, 1966, p. 881.
7. Manch, W. and Fernelius, W. C., *J. Chem. Educn.*, 1961, 38, 192.
8. Maki, G., *J. Chem. Phys.*, 1958, **29**, 1129.

DETERMINATION OF MANGANESE BY NEUTRON ACTIVATION ANALYSIS USING Am^{241}-Be SOURCE

EVEN-THOUGH the neutron flux that can be obtained from the isotopic neutron sources is small ($\approx 10^4 n$/cm^2–sec), compared to reactor flux (10^{12}-10^{15} n/cm^2-sec), they have other advantages like easy transportability, low cost, no health hazard and highly constant flux. Due to these advantages, isotopic neutron sources are ideal for routine non-destructive analysis of quite a large number of elements such as Mn, La, Cu[1], V, I, Br, Ag, etc. Manganese is present in small amounts in ilmenite and its determination by chemical methods is tedious and time consuming. However, it can easily be determined by neutron activation analysis using isotopic neutron sources. In this paper, a simple procedure developed by us for the routine activation analysis of manganese present in small quantities in ores is reported. The analysis is based on the $Mn^{55}(n, \gamma) Mn^{56}$ reaction using tehrmal neutrons from the Am^{241}–Be source and counting the 0·84 MeV. photopeak of Mn^{56} ($T_{\frac{1}{2}} = 2 \cdot 58$ hrs.) formed.

Experimental and Results

To 5 gm. of finely powdered ilmenite, varying amounts (0·05 to 0·25 gm) of pure MnO_2 (L.R., B.D.H.) are added, mixed well and packed in polythene vials of identical size (5 ml) and shape. They are then suspended by means of a cotton thread in the Am^{241}–Be neutron source (supplied by Electronics Corporation of India Limited) at the position of maximum flux. After irradiating for 15 hours the samples are counted for 10 min. period using the 400 channel gamma ray pulse height analyser (Frieseke and Hoepfner). In each case, the counting is done 4 or 5 times and the statistical average of counts are taken.

Mn^{56} gives photopeak of 0·84 Mev. The fractional photopeak areas are calculated (by Covell's method[2]) as follows. Let a_0 be the channel response at the maximum of the photopeak, a_1, a_2, ... a_n are the channel responses on the left side of a_0, and b_1, b_2, b_n are the channel responses on the right side of a_0. The area "N" of the peak above the line joining a_n and b_n bears a constant proportionality to the gamma ray abundance and hence to the amount of element present in the sample. This fractional area N can be calculated from the expression

$$N = a_0 + \sum_1^n a_i + \sum_1^n b_i - (n + \tfrac{1}{2})(a_n + b_n)$$

The area "N" is calculated for each sample. This is plotted against the amount of manganese added to the ore in the standard samples prepared.

A straight line plot (Fig. 1) is obtained. The intercept corresponds to the counts due to manganese present in pure ilmenite. From the graph, the amount of manganese present in ilmenite is read. The values obtained for the determination of manganese in ilmenite are presented in Table I.

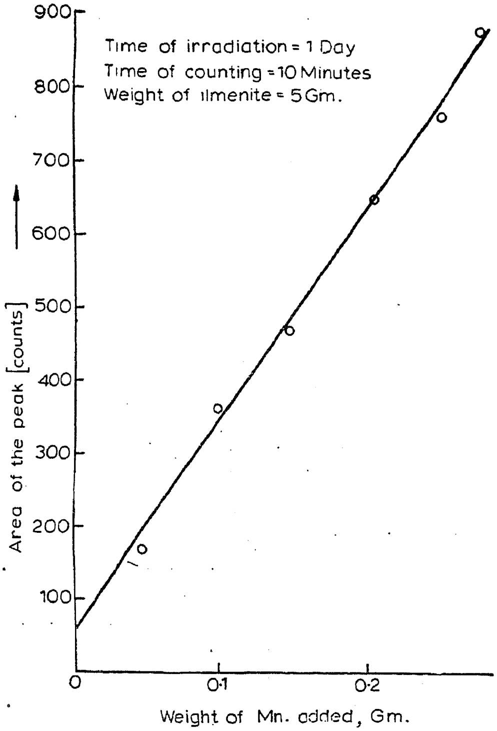

FIG. 1. Ilménite.

TABLE I

Determination of Mn by neutron activation analysis

Experiment No.	% Manganese found
1	0·35
2	0·35
3	0·40

The analytical value for manganese in the ilmenite is 0·37%.

It is clear from the above results that accurate and reproducible results can be obtained for the determination of manganese even if present in small quantities, by neutron activation analysis using isotopic neutron sources.

The authors wish to thank Prof. J. C. Kuriacose, Head of the Department of Chemistry, for his keen interest in this work.

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---

1. Rao, V. R. S. and Sudhakar. Ch., "Determination copper in coordination complexes using a low flux neutron source," Paper in print; *Journal of Radiochem. Radioanal. Letters.*
2. Covell, D. F., *Anal. Chem.*, 1959, 31, 1785.

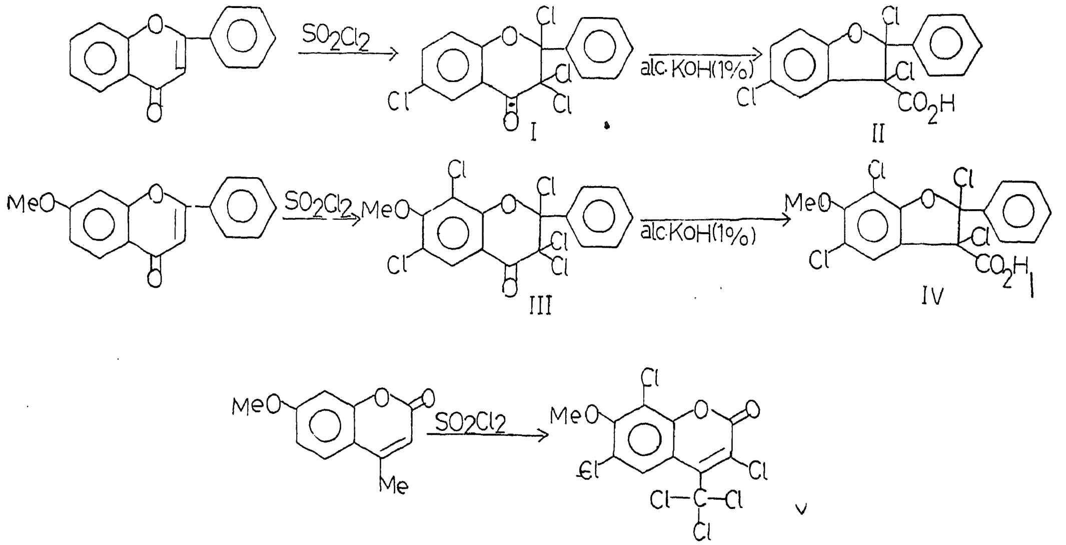
SULPHURYL CHLORIDE—SULPHOLANE A POWERFUL CHLORINATING AGENT

IN connection with our work on the halogenation of benzopyrone derivatives we investigated the reactions of some flavone and coumarin derivatives with sulphuryl chloride in different solvents. Whilst flavone (1 g.) on heating with excess of sulphuryl chloride (20 ml.) in benzene or carbon tetrachloride solution for 15 hour has been reported[1] to yield 2, 3, 3-trichloroflavanone, the same reaction in sulpholane gives a tetrachloroflavanone derivative, m.p. 180–82°. The latter has been assigned the structure as 2, 3, 3, 6-tetrachloroflavanone (I) on the basis of its analytical-spectral data and chemical properties. The i.r. spectrum of (I) showed a band around 1720 cm^{-1} indicating α, β-substitution whereas the n.m.r. spectrum gave signals for only aromatic protons around 6–8·2 δ.

The same compound was also obtained when 6-chloroflavone was heated with excess of sulphuryl chloride in benzene solution. The structure of (I) was further confirmed by its hydrolysis with alcoholic KOH to afford the previously reported[2] trichlorocarboxylic acid (II). The use of sulpholane for higher halogenation was also observed in the case of 7-methoxyflavone when a pentachloroflavanone derivative (III), m.p. 185–86° was isolated whilst other solvents under similar conditions yielded di-, tri- and tetra-chloro derivatives[1b].

The structure (III) was fully consistent with its analytical and spectral data. Its i.r. (KBr) showed a $C = O$ band around 1760 cm^{-1} whilst ether bands were obtained at 2850, 1250 and 1020 cm^{-1}. The n.m.r. spectrum ($CDCl_3$) showed a singlet at 4·0 δ corresponding to $-OCH_3$ protons whereas the aromatic protons were obtained as a multiplet in the region 6 to 8·2 δ. On alcoholic alkaline hydrolysis (III) yielded a tetrachloro acid IV, m.p. 145–46°, an observation in complete agreement with our previous findings[2].

We next investigated the reaction of coumarin with sulphuryl chloride in sulpholane medium. In this case 3, 6-dichlorocoumarin, m.p. 163–64°



(Reported[3] m.p. 167–68°) was isolated. Its structure was established by its alkaline $KMnO_4$ oxidation to give 5-chlorosalicylic acid, m.p. 166–67°, (Reported[4] m.p. 167–68°). It is of interest to mention that halogenation of coumarin with sulphuryl chloride in carbon tetrachloride gave only 3-chlorocoumarin, m.p. 118–19° (Reported[5] 122–23°). The higher halogenation power of sulphuryl chloride-sulpholane medium was also evident in the reaction with 7-methoxy-4-methylcoumarin when a hexachloro compound, m.p. 150–53° was obtained which from its analytical and spectral data was assigned the structure (V).

It can thus be seen that sulphuryl chloride in sulpholane is strong chlorinating agent, and may be utilized for the preparation of polyhalogenated compounds.

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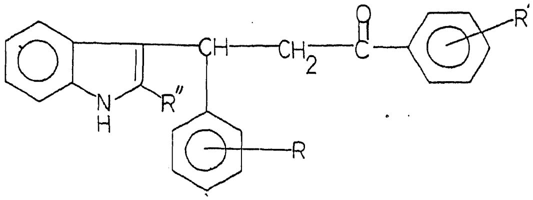
1*a*. Merchant, J. R. and Rage, D. V., *Chemical Comm.*, 1970, p. 380.
b. — and —, *Tetrahedron*, 1971, 27, 4837.
2. Bhat. A. R., *Ph.D. Thesis*, University off Bombay, 1971.
3. Corre, M. L. and Levas, E., *Compt. Rend.*, 1963, 257 (9), 1622; *C.A.*, 1963, 59, 12744 *h*.
4. Weil, H., Traun, M. and Marcel, S., *Ber.*, 1922, 55, 2664.
5. Perkin, W. H., *J. Chem. Soc.*, 1871, 24, 44.

SOME LESSER KNOWN REACTIONS OF CHALKONES

THE present communication describes some of th less known reactions of chalkones which have nc been studied in detail by previous workers[1-5].

Carboxy chalkones are best prepared by the alka line hydrolysis of the corresponding cyanochalkone: Thus, 4-fluoro-4′-cyanochalkone (I), m.p. 160°, pre pared by the condensation of *p*-cyanoacetophenon (II) and *p*-fluoro-benzaldehyde in 10% NaOH an 4-bromo-4′-cyanochalkone, m.p. 169–70°, obtaine by the condensation of II with *p*-bromobenzaldehyd yielded on boiling with 10% alkali for 5 hours th corresponding carboxy chalkones, m.p. 165° (fron water) and 130° (from dil. alcohol) respectively.

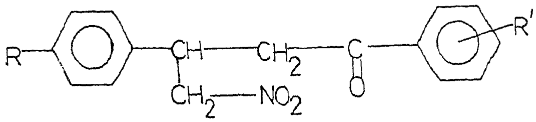
The reaction of the chalkone I with indole an that of 4′-cyano-3-methoxychalkone, m.p. 133° wit 2-methylindole in presence of a mixture of aceti acid and acetic anhydride afforded 60% yield o the adduct having the structures[1] (A).



(A)

R = 4–F; R′ = 4–CN; R″ = H, m.p. 172° (benzene)
R = 3–OMe; R′ = 4–CN; R″ = CH_3, m.p. 195 (benzene).

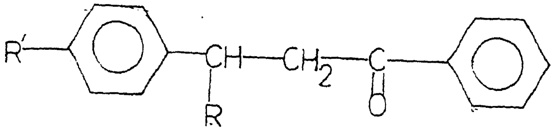
The reaction of 4-chloro-3′, 4′-dimethoxychalkone, m.p. 118° and 3′ 4′, 4-trimethoxychalkone, m.p. 85° with nitromethane in methanolic solution in presence of sodium gave adducts of the following general structure[2,3] (B).



(B)

$R = 4\text{-}Cl$, $R' = 3', 4'\ (OMe)_2$ (benzene), m.p. 120°.
$R = 4\text{-}OMe$, $R' = 3'\ 4'\ (OMe)_2$ (benzene) m.p. 118°.

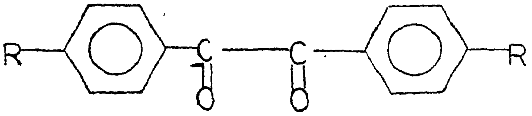
When 4-methoxy- and 4-methyl- chalkones were treated with methyl magnesium iodide, compounds of the general structure (C) shown were obtained[4]. The i.r. spectra showed a carbonyl band around 1685 cm^{-1}.



(C)

$R = CH_3$; $R' = 4\text{-}OCH_3$, m.p. 72° (dil. alcohol).
$R = CH_3$; $R' = 4\text{-}CH_3$, b.p. 200–205°/2 mm.

The oxidation of 4-methyl and 4′-methyl chalkones with thallium (III) nitrate in presence of glyme and perchloric acid (70%) afforded diketones of the structure[5] shown in (D).



(D)

$R = 4\text{-}CH_3$, m.p. 136–37° (yellow needles, from alcohol).
$r = H$, $R' = 4\text{-}CH_3$, m.p. 140° (colourless needles from alcohol).

All compounds gave satisfactory analysis for C, H and N wherever present.

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1. Salgar, S. S., Chhatriwala, K. M. and Merchant, J. R., *Curr. Sci.*, 1966, 35, 123.
2. Kohler, E. P., *J. Am. Chem. Soc.*, 1916, 38, 889 ; 1924, 46, 503.
3. Kloetzel, M. C., *Ibid.*, 1947, 69, 2271 ; Fishman, N. and Zubfanti, S., *Ibid.*, 1951, 73, 4466 ; Davey, W. and Tivey, D. J., *J. Chem. Soc.*, 1958, p. 227.
4. Lespaguol, R. F., Lespaguol, A. and Cazin, C., *Bull. Soc. Chim.* (France), 1964, 7, 1507 ; *C.A.*, 1965, 62, 5212.
5. Mckittop, A. and Curtrus, T. E., *Tatrahedron Letters*, 1970, p. 5281.

ISOSCUTELLARIN AND OTHER POLYPHENOLS FROM THE LEAVES OF *STERCULIA FOETIDA*

Sterculia foetida L. (family : Sterculiaceae) recorded[1] to contain a mitogenic principle, sterculic acid present as its methyl ester has not been systematically examined for its polyphenolic components. In continuation of our isolation of 6-O-β-D-glucuronyl luteolin from *S. colorata*[2], we have examined the leaves and flowers of *S. foetida*, and the results are recorded here.

Fresh leaves were extracted with hot 95% EtOH under reflux and the residue in the concentrate was fractionated by solvent extraction. The ethyl acetate as well as ethyl methyl ketone extracts on concentration yielded a creamy white solid, not melting below 320° (with blackening at about 220°). It was insoluble in ether and acetone, soluble in MeOH, laevorotatory, λ_{max} 280 nm and gave an olive green colour with Fe^{3+}. It developed a pink colour with hot HCl, and on hydrolysis (3N HCl in MeOH medium, 1 hr) yielded cyanidin chloride and D-glucuronic acid. It also underwent hydrolysis with β-glucuronidase. The compound was thus identified as a procyanidin-β-D-glucuronide.

The mother liquor, after the removal of the procyanidin, on PC indicated the presence of two flavone glycosides. They were separated by fractional crystallisation ($MeOH\text{–}Me_2CO$) followed by preparative PC. The major component (more soluble in $MeOH\text{–}Me_2CO$) had no sharp m.p. (decomposition started from 230°), was purple under U.V. and U.V/NH_3, gave olive green colour with Fe^{3+} and yellow with alkalis. It had λ_{max} (nm) 249 sh., 268, 340 (MeOH) ; 280, 322 sh., 375 (NaOAc) and 265, 345 ($NaOAc/H_3BO_3$) ν_{max} (KBr) 3400 (br., multiple OH), 2910, 1715 ; 1650 (carbonyl), 1610, 1570, 1500, 1440 (aromatic ring) 1380 ; 1300, 1255, 1210, 1185 (phenolic OH) ; 1140, 1105, 1060, 1050, 1020 ; 1000, 925 (glycoside moiety), 845, 800 and 760 cm^{-1} (substituted benzene) and R_f : (×100, Whatman No. 1, ascending 27 ± 2°) 54 (H_2O), 22 (15% HOAc), 42 (30% HOAc), 61 50% HOAc), 35 (BAW), 62 (phenol), 72 (Forestal) and 53 (*t*-BAW). On acid as well as enzyme (β-glucuronidase) hydrolysis, it yielded scutellarein (4′, 5, 6, 7-tetrahydroxy flavone) and D-glucuronic acid in equal molar ratio. Hence, it was identified as a scutellarein mono-glucuronide. From the colour reactions, UV fluorescence and spectral data, glycosylation was taken to be at A-ring and most probably at C–6. A direct comparison of the compound with scutellarin (7-O-β-D-glucuronyl scutellarein) (IR, R_f and co-PC) showed them as different and hence the new glycoside was identified as 6-O-β-D-glucuronyl scutellarein, designated isoscutellarin. The other minor glycoside was identified as 6-O-β-D-glucuronyl luteolin by λ_{max}, R_f, acid and enzyme hydrolysis and direct comparison including co-PC with an authentic sample from *S. colorata*[2].

The purple coloured flower petals were extracted with 0·01 N methanolic HCl and concentrated *in vacuo* below 40° C. The dark purple solid was crystallised from methanolic HCl-ether. It was homogenous on PC, did not melt upto 320°, and yielded cyanidin chloride and glucose on hydrolysis. It was identified as cyanidin-3-O-glucoside by R_f and co-PC with an authentic sample.

This is the first record of occurrence of 6-O-β-D-glucuronyl scutellarein, an isomer of scutellarin; *S. foetida* containing isoscutellarin and 6-O-β-D-glucuronyl luteolin resembles *S. colorata* containing the latter with scutellarein but differs from it in having significant quantity of the procyanidin glucuronide. The presence of 6-oxygenated flavones in *Sterculia* sp. is significant from the point of molecular taxonomy of the Natural Order Malvales.

We thank the Regional Sophisticated Instrumentation Centre, Indian Institute of Technology, Madras, for the spectral data, the University Grants Commission, New Delhi, for a research grant and the Principal of our Institute for encouragement.

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---

1. Scarpelli, D. G., *Science*, 1974, 185, 958.
2. Nair, A. G. R., Ramesh, P. and Subramanian, S. S., *Phytochem.*, 1976 (in press).

TOXICITY OF *PARTHENIUM HYSTEROPHORUS* L.

Parthenium hysterophorus L., an alien weed now growing wild in many parts of India is posing agricultural and health hazards. It has been shown to cause allergic contact dermatitis in humans[1,2]. Agriculturists in Maharashtra and Karnataka are expressing concern over the invasion of food and fodder crop fields by this weed. Since livestock grazing in open fields have an access to the weed it is of interest to know whether *Parthenium* has any after effects after ingestion. We have undertaken a detailed investigation on the toxicology of *Parthenium* to animals and the present report describes our initial findings on the toxicity of the weed to livestock.

During our field survey it was found that goats graze on *P. hysterophorus* freely while cattle and buffaloes appeared to graze sparingly. Under laboratory conditions we found that both buffaloe bull calves and cross bred bull calves freely feed on *Parthenium* either as such or after admixture with conventional green fodder. When nine buffaloe bull calves and seven cross bred bull calves were fed on the weed mixed with green fodder *ad libitum*, they developed toxic symptoms resulting in the death of six buffaloe bull calves and five cross bred bull calves within periods ranging from 8 to 30 days after feeding. All the buffaloe calves developed severe dermatitis on face, base of the ears, shoulders, neck, back, loins and on legs extending down to hock and knee joints. Autopsy of the animals revealed gross and microscopic lesions in the gastro-intestinal tract, liver and kidney. Control animals fed on conventional green fodder remained healthy and showed none of these external symptoms.

It is increasingly realized that parthenin, the major sesquiterpene lactone present in *P. hysterophorus* is responsible for allergic contact dermatitis of humans by this weed[3]. It is also reported that sesquiterpene lactones from certain members of Compositae family to which *Parthenium* also belongs, are toxic to livestock[4]. In our present studies it was found that subconjunctival and intracorneal tests[5] with an aqueous solution of parthenin (0·1% w/v) showed that the dermatitis manifested by *Parthenium*-fed animals was of allergic nature involving Type IV hypersensitivity. Further experiments to assess the possibility of excretion of parthenin and other toxic principles from the injested weed by livestock through milk and their impact on human health are under way. The details of these studies will be published elsewhere.

We thank Prof. Satish Dhawan, Director, Indian Institute of Science, and Mr. M. A. Sethu Rao, Officiating Secretary, Karnataka State Council for Science and Technology for their keen interest. The help rendered by Bangalore City Corporation authorities in the supply of *Parthenium* is gratefully acknowledged.

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---

1. Lonkar, A., Mitchell, J. and Calnan, C. D., *Trans. St. John's Hosp. Derm. Soc.*, 1974, 60, 43.
2. Subba Rao, P. V., Mangala, A., Subba Rao, B. S. and Prakash, K. M., *Proc. Soc. Biol. Chem. (India)*, 1976, 45, 12.
3. Mitchell, J. C., *Recent Adv. Phytochem.*, 1975, 9, 119.
4. Kingsbury, J. M., *Poisonous Plants of the United States and Canada*, Prentice-Hall, Englewood Cliffs, New Jersy, U.S.A., 1964, p. 409.
5. Herbert, W. J., *Veterinary Immunology*, Blackwell Scientific Publications, Oxford, England, 1970, p. 83.

PRELIMINARY OBSERVATIONS ON THE PRODUCTION OF AMYLOGLUCOSIDASE BY THERMOPHILIC FUNGI

KERR *et al* (1941) and Underkofler (1953) among others recognised that enzymes of amyloglucosidase type were present in preparations which produced fermentable sugars from starch. Philips and Caldwell (1951), Arima (1964), Nehira and Nomi (1956), Aschengreen (1969), Underkofler (1969), Mangallam (1973) and others studied the production of amyloglucosidase by species of *Aspergilli* and *Rhizopus*. Thus, in literature several reports of fungi producing this enzyme are available but none deals with thermophilic fungi. Therefore, a preliminary investigation on the production of amyloglucosidase by thermophilic fungi was carried out, with the hope to isolate a thermostable enzyme.

Four thermophilic fungi *viz.*, *Torula thermophila* Apinis; *Mucor miehe* Cooney and Emerson; *Humicola lanuginosa* (Griffon and Maublanc) Bunce; *Sporotrichum thermophile* Cooney and Emerson, which are unable to grow below 25° C and grow well above 50° C, thus satisfying the definition of Cooney and Emerson (1964) were screened for amyloglucosidase activity in the following manner.

100 ml. of the seed medium of the following composition (Tapioca starch 1%, CSL 1%, NH_4NO_3 0·25%, KH_2PO_4 0·2%, $MgSO_4$ 0·1%, pH 6) was distributed in 500 ml. Erlenmeyer flasks, plugged with non-absorbent cotton and sterilised for 45 mts., at 120° C and 15 lb./sq. in. pressure. After cooling they were inoculated with 2·5 ml. fungal spore suspension prepared from five-day old, heavily sporulating cultures grown on potato dextrose agar slants. They were incubated at 50° C for 48 hrs. on a rotary shaker with 280 r.p.m. and 2" throw.

Production of enzyme: Sterilized 500 ml. Erlenmeyer flasks containing 100 ml. production medium (Composition: Tapioca starch 2·5%, Peanut meal 2%, ammonium nitrate 0·5%, KH_2PO_4 0·25%, K_2HPO_4 0·2%, $MgSO_4$ 0·1%, pH 6·5) were inoculated with 2·5 ml. seed. Th mold was grown for 96 hrs. At the end of th fermentation cycle the mycelium of each speci was separately harvested and filtered. The cultu filtrate was assayed and quantitative estimation o the enzyme in terms of glucose was carried out b Hane's method (1947). The conversion of starc to glucose by the enzyme was first identified b preparing glucosazone with phenyl hydrazine a later confirmed by paper chromatography. Th enzyme is found to be stable for 60 mts. at 60° Mean of triplicate results are given in Table I.

TABLE I

Amyloglucosidase production by thermophilic fung

Sl. No.	Organism	Temp. (° C)	Age (Hrs)	pH	Filtrate activity* μ/ml
1.	*Terula thermophila*	50	96	7·0	195·1
2.	*Mucor miehe*	50	96	6·7	102·
3.	*Humicola lanuginosa*	50	96	7·0	75·
4.	*Sporotrichum thermophile*	50	96	6·7	149·

* One amyloglucosidase unit is defined as the am of enzyme releasing 1 mg. of glucose in one hour f a 4% starch solution at pH 4·5 at 60° C.

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---

1. Arima, K., In *Global Impacts of Microbio* Ed. by Starr, John Wiley and Sons, N York, 1964, p. 277.
2. Aschengreen, N. H., *Process Biochemi* 1969, 4, 23.
3. Cooney, D. G. and Emerson, R., *Thermop Fungi*, Freeman, San Francisco, Califo 1964, p. 188.
4. Kerr, R. W., Meisel, H. and Schink, N. *Ind. Eng. Chem.*, 1941, 33, 1418.
5. Mangallam, S., *Ph.D. Thesis*, Poona Uni sity, Poona, India, 1973.
6. Nehira, T. and Nomi, R., *J. Ferm. Tech* (Japan), 1956, 34, 391.
7. Hanes, C. S., *Practical Physiological Chemi* Ed. Philips, B. H., Publ. Blackstein Co., I New York, 1947, p. 1.

HAEMATOLOGICAL STUDIES ON THE RED COTTON BUG, *DYSDERCUS KOENIGII*

THIS communication reports changes in total haemocyte count, differential haemocyte count and haemolymph volume during the maturation period of *Dysdercus koenigii*.

The bugs were reared in the laboratory at 28 ± 1° C and 5 insects were sacrificed at 24 hour interval for a period of five days and the haemolymph volume of the insects was measured by amaranth dye dilution method (Mordue and Goldsworthy)[1]. Total haemocyte count (THC) per mm^3 at different time intervals in male and female insects was calculated using the method of Wheeler[2], Jones'[3] classification was used for identification of haemocytes. However, for differential haemocyte count (DHC—prohaemocytes (germinal cells) and plasmatocytes (phagocytic cells) only were taken into consideration. Wheeler's[2] method was adopted for estimating the differential haemocyte count. Absolute number of haemocytes was calculated by combining the data of total haemocyte count and haemolymph volume.

TABLE I

*Comparative haemocyte count, differential count and absolute number of adult insects**

Age of insect	Sex	Weight of insect in mg	THC per mm^3	DHC in %		Haemolyph volume in μl	Absolute No. of haemocytes
				Prohae-mocytes	Plasmato-cytes		
Just emerged	Male	50	12,000± 500	30	70	14±0·20	1,68,000
	Female	90	16,000±1500	30	70	16±1·0	2,56,000
1 day old	Male	60	11,000± 200	35	65	15±0·32	1,65,000
	Female	100	14,000±1000	35	65	18±0·50	2,52,000
2 days old	Male	80	10,500± 200	38	62	16±1·00	1,68,000
	Female	120	12,000± 500	38	62	20±1·00	2,40,000
3 days old	Male	80	10,500± 300	33	67	16±0·50	1,63,000
	Female	150	12,500± 200	33	67	28±1·50	3,50,000
4 days old	Male	90	10,000± 500	40	60	18±0·30	1,80,000
	Female	190	13,000± 450	40	60	32±1·50	4,16,000
5 days old	Male	100	10,000± 350	48	52	18±1·00	1,80,000
	Female	200	13,000± 400	48	52	38±1·50	4,94,000

* Mean of five insects in all the experiments.

The results are tabulated in Table I. In males, the blood volume showed gradual increase for the first two days and then again the 4th day, *viz.*, the mating day. In female, from 1st to 5th day there was a steady increase in the blood volume and the rate of increase was much more pronounced than in males. In both males and females, the haemolymph accounted for 18% to 20% of the total body weight (*see* Table I).

The total haemocyte count was more or less steady throughout the 5 days. In both males and females, the prohaemocytes were always less than plasmatocytes. However, slight variations were noted (*see* Table I).

In males, the absolute number of haemocytes does not show much variation during maturation. However, in females, a rise in the absolute number of haemocytes was observed on the 3rd day which reaches its maximum on the 5th day.

A comparison of the total haemocyte count of the red cotton bug, with that of *Oncopeltus fasciatus*[4] shows that *Dysdercus koenigii* has a relatively high total haemocyte count.

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1. Mordue, W. and Goldsworthy, G. J., *Gen. Comp. Endocrinol.*, 1969, 2, 360.
2. Wheeler, R. E., *J. Insect Physical.*, 1963, **9**, 223.
3. Jones, J. C., *Biol. Bull.*, 1965, 129 (2), 282.
4. Feir, D., *Nature*, London, 1964, 202, 1136.

FURTHER OBSERVATIONS ON OVIPOSITION OF *DIMARMUS VAGABUNDUS* TIMB. (PTEROMALIDAE: HYMENOPTERA)

Dimarmus vagabundus Timberlake is a common parasite of the larvae of different species of genus *Callosobruchus*[1,2], the notorious pests of stored pulses. The oviposition behaviour of this parasite has already been studied but contradictory statements have been made on the laying of eggs[3,4]. According to Chatterji[3] the female lays its eggs on the mature embroyos of the host before hatching of the eggs. Cheema and Misra[4], on the other hand, noted the female to insert its ovipositor through the larval hole of the host and lay the eggs on the surface of the larva within the seed. They further noted that the eggs laid at a time when bruchid larvae are very young, fail to complete development.

Detailed observations made on the oviposition behaviour of *D. Vagabundus* show that normally the female penetrates its sharp and pointed ovipositor through the surface of the seed and places the egg on the surface of the growing larva of the bruchid in the seed. The female sometimes inserts her ovipositor through the hole made by the bruchid larva, for the deposition of eggs. It is thus apparent that observations of Chatterji about the penetration by the ovipositor of the female is quite correct but this author failed to appreciate the time of oviposition and therefore, supposed the egg to be laid on the surface of the embryo. It may however, be added that such a mode of oviposition is rarely adopted by the female. The most common method of oviposition instead, constitutes the drilling of the tissue of the seed for reaching the contained larva. It has also been observed that the complete process of laying an egg in this manner requires about 2–3 minutes when the larva is fully grown and 5–7 minutes when the larva is at an early stage of development. The fully grown larvae were given distinct perference for oviposition.

With regard to the egg laying capacity of a female, oviposition data at different conditions of temperatures and relative humidities indicate that the number of eggs per female varies from 9 to 26 (Table I). Likewise the oviposition period under similar conditions ranged from 2·7 to 6·7 days.

TABLE I

*Average number of eggs laid and *average oviposition period of one female (ten observaions) under different conditions of temperature and relative humidity*

Relative Humidity	25° C	30° C	34° C	38° C
30%	9·0 (6·4)	18·4 (3·9)	20·6 (2·8)	8·8 (2·7)
50%	12·4 (6·6)	20·5 (4·0)	22·0 (3·2)	12·2 (2·7)
70%	14·0 (6·7)	25·0 (4·2)	26·4 (3·4)	9·8 (3·0)

* Average oviposition period shown within brackets

The parasite is thus a good agent for the biological control of bruchids but its limited fertility does not match the high rate of reproduction exhibited by the bruchids.

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1. Ferriera Lima, A. D., *Bol. Soc. Brasil*, Agron. 1942, 5, 441.
2. Mani, M. S., *Ind. J. Ent.*, 1939, 1, 69.
3. Chatterji, S., *Ibid.*, 1954, 16, 77.
4. Cheema, P. S. and Misra, J. N., *Curr. Sci.*, 1962, 31, 21.

NITROGEN CONTENT OF RHIZOSPHERE SOILS OF WILD WEEDS AND CULTIVATED RICE

RHIZOSPHERES of certain weeds and rice plants have been shown to possess demonstrable nitrogenase activity as revealed by the acetylene reduction method[1-5]. Apart from the inherent limitations of the method, research workers have often used data from laboratory estimations of nitrogenase activity of small samples for the calculation of nitrogen accretion in terms of kg N/ha. It was therefore considered worthwhile to find out the total nitrogen status of rhizosphere soils of some wild weeds and varieties of cultivated rice by means of a conventional procedure. Samples of rhizosphere and non-rhizosphere soils (10 g. each) from several weeds were collected from uncultivated fields in rural areas of Delhi. The number of aerobic bacteria capable of growing on Jensen's nitrogen-free medium was estimated by plating dilutions of soil samples and the total nitrogen was determined by Kjeldahl's method. In a similar way, samples of rhizosphere and non-rhizosphere soils of different varieties of rice from various places in India, from plots which had not received any inorganic source of nitrogen, were also collected and analysed for total nitrogen.

The amount of nitrogen in the rhizosphere varied, although seven weeds had more nitrogen in the rhizosphere than in the non-rhizosphere. However, the amount of nitrogen in the rhizosphere could not be correlated with the number of bacteria which could be grown on a nitrogen free medium (Table I).

Among the 20 plots sampled for rice, 10 showed increase in nitrogen content in the rhizosphere and the maximum accretion was observed with the

TABLE I

Total nitrogen content and population of bacteria capable of growing on nitrogen-free Jensen's medium in the rhizosphere soils of several wild weeds (mean values of duplicate samples)

Locations	Sl. No.	Name of the weed	N mg/100 g soil	Bacteria capable of growing on N free medium 10^3/g (Figures in parenthesis indicate R : S ratios)
1	2	3	4	5
A	1	Non-rhizosphere (control)	84	26·6
	2	*Cyperus difformis* (Cyperaceae)	101	44·0 (1·65)
	3	*Scirpus* sp. (Cyperaceae)	72	21·0 (0·79)
B	4	Non-rhizosphere (control)	99	26·3
	5	*Ammania sevegalensis*	65	17·6 (0·67)
	6	*Solanum xanthocarpus*	172	44·0 (1·67)
C	7	Non-rhizosphere (control)	134	16·3
	8	*Croton sparsiflorus* (Euphorbiaceae)	99	20·5 (1·25)
D	9	Non-rhizosphere (control)	67	17·3
	10	*Xanthium stumarium* (Compositae)	226	18·3 (1·05)
E	11	Non-rhizosphere (control)	40	18·3
	12	*Asphodelus tenuifolius* (Liliaceae)	32	22·9 (1·25)
	13	*Vicia sativa* (Leguminosae)	34	23·6 (0·88)
	14	*Trigonella incisa* (Leguminosae)	32	31·9 (1·19)
	15	*Spergula arvensis* (Caryophyllaceae)	29	45·7 (1·71)
	16	*Fumaria indica* (Fumoroideae)	32	25·2 (0·94)
	17	*Sisymbrium iris* (Cruciferae)	58	40·6 (1·52)
	18	*Cenchrus ciliaris* (Gramineae)	74	94·0 (3·52)
F	19	Non-rhizosphere (control)	43	4·6
	20	*Parthenium hysterophorus* (Compositae)	46	5·2 (1·13)
G	21	Non-rhizosphere (control)	38	4·3
	22	*Eclipta alba* (Compositae)	40	3·0 (0·69)
H	23	Non-rhizosphere (control)	48	61·0
	24	*Tephrosia purpurea* (Leguminosae)	32	0·3 (—)
	25	*Ocimum americanum* (Labiatae)	30	2·3 (0·037)
I	26	Non-rhizosphere (control)	51	2·9
	27	*Dichanthium annulatum* (Gramineae)	47	3·9 (1·34)
J	28	Non-rhizosphere (control)	37	9·0
	29	*Leucas cephalotes* (Labiatae)	33	4·0 (0·44)
K	30	Non-rhizosphere (control)	53	17·3
	31	*Convolvulus arvensis* (Convolvulaceae)	36	9·3 (0·53)

variety 'Madhu,' at Bangalore (Table II). Depletion of nitrogen in the rhizosphere was characteristic of other varieties from the remaining 10 plots and the maximum decrease was observed in the rhizosphere soil of Sona variety at Delhi.

Earlier workers have pointed out consistent increase in the nitrogenase activity in the rhizosphere of weeds and rice but our results indicate that accumulation of nitrogen in the rhizosphere was not a consistent feature, the reason for which may be denitrification processes in the rhizosphere, the inability of bacteria to fix nitrogen in the rhizosphere or the uptake of nitrogen by plants.

TABLE II

Total nitrogen content in rhizosphere (R) and non-rhizosphere (NR) soils of rice plants from various places in India from plots which had not received inorganic N fertilizer (mean of 2 or more replicate samples, 10 g each)

Sl. No.	Place	Variety	Age in days	N (mg/100 g soil)		
				R soil	NR soil	Difference between R and NR soils
1.	Cuttack	Supriya	53	77	64	+ 13
2.	Cuttack	Supriya	120	69	61	+ 8
3.	Pantnagar	IR–24	40	108	98	+ 10
4.	Pantnagar	Pusa	20	102	102	0
5.	Karnal	2–21*	15	44	47	— 3
6.	West Bengal	Local	60	82	73	+ 9
7.	Delhi	Sona*	60	84	181	— 97
8.	Delhi	Sona*	120	100	81	+ 19
9.	Deoria	Jaya*	90	38	69	— 31
10.	Varanasi	Sona*	90	56	69	— 13
11.	Varanasi	Ratna*	90	56	62	— 6
12.	Jabalpur	..	..	40	26	+ 14
13.	Bangalore	Madhu*	40	206	88	+118
14.	Lucknow	T_9	30	44	47	— 3
15.	Lucknow	Saket 4	45	41	56	— 15
16.	Lucknow	T_{26}	45	67	62	+ 5
17.	Lucknow	Saket 4	60	64	78	— 14
18.	Hyderabad	Rajhansa	90	97	88	+ 9
19.	Hyderabad	5 varieties	100	73	50	+ 23
20.	Trivandrum	Jaya	110	149	212	— 63

* Single sample.

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---

1. Dommergues, Y., Balandreau, J., Rinaudo, G. and Pierrette, W., *Soil Biol. Biochem.*, 1973, 5, 83.
2. Hardy, R. W. F., Burns, R. C. and Holstein, R. D., *Ibid.*, 1973, 5, 47.
3. Harris, D. and Dart, P. J., *Ibid.*, 1973, 5, 277
4. Rinaudo, G., Balandreau, J. and Dommergues Y., *Pl. Soil Sp, Vol.*, 1971, p. 471.
5. Yoshida, T. and Ancajao, R. R., *Soil Sci. Soc Amer. Proc.*, 1971, 35, 156.

PECTIC AND CELLULOLYTIC ENZYMES ASSOCIATED WITH *GLOEOSPORIUM* ROT OF PAPAYA

PAPAYA (*Carica papaya* L.) fruit rot caused by *Gloeosporium papayae* P. Henn. was examined, to find out the involvement of pectic and cellulolytic enzymes in the disease.

Extracts of healthy and infected tissue were prepared by homogenising 100 g of the tissue in 100 ml of distilled water. Homogenised tissue extract was centrifuged for 5 minutes at 3000 r.p.m. Clear liquid thus obtained was used as enzyme extract for enzyme analysis. Pathogen was also grown in natural papaya pulp medium, to determine secretion of these enzymes. Water extract of the above, after removing fungal growth was used as the source of enzyme.

The following enzymes were detected during the studies. Polygalacturonase (PG), Polymethylgalacturonase (PMG), Pectinmethyl- trans- eliminase (PMTE), Polygalacturonate- trans- eliminase (PGTE), Pectin Methylesterase (PME) and Cellulase. The substrate for PG and PGTE was sodium polypectate; for PMG, PMTE and PME, pectin; and for cellulase, carboxy methylcellulose. Reaction pH for glycosidases was 4·6; for transeliminases 8; and for cellulase 5. PG, PMG, PGTE, PMTE, and cellulase are viscosity reducing enzymes and were determined by viscometric procedure (Van Etten *et al.*, 1967)[1]. PME was determined by continuous titration method (Kertesz, 1951)[2]. Boiled enzyme extracts were taken as control which did not show any enzyme activity.

TABLE I

Pectic and cellulolytic enzymes associated with healthy and infected papaya fruit, and secreted in papaya pulp medium by the pathogen

	Days of incubation	Enzyme Activity*					
		PG	PMG	PME	PGTE	PMTE	Cellulase
Healthy Fruit	7	5·2	..	0·033	..	..	..
	12	11·5	..	0·044	..	..	..
Infected Fruit	7	71·4	47·9	0·077	10·4	17	27·3
	12	43·4	17·9	0·102	22·5	37·7	36
Papaya Pulp Medium	4	49	53	..	10·3	6	..
	7	18·6	16·2	..	25·6	22	8·1
	9	13·9	9·0	..	32·3	34	11·8
	12	..	..	..	21·0	17·7	4·5

* Enzyme activity of PG, PMG, PGTE, PMTE and Cellulase, expressed as the % loss in the viscosity of enzyme-substrate reaction mixture after 90 min reaction time. Activity of PME expressed as the amount of 0·22 N NaOH in ml., absorbed/ml enzyme-substrate reaction mixture during 120 min. reaction time. Total volume of the reaction mixture for PME was 35 ml; 5 ml enzyme extract, 30 ml 1·2% pectin in water. Values of enzyme activity are the mean of three separate observations for each.

Results have been presented in Table I. Out of the various enzymes studied, extracts of just ripe healthy papaya tissue, indicated the activity of PME and PG. PME was not secreted by the pathogen in papaya pulp medium, but its activity was detected in the extracts of infected tissue. It may therefore be concluded that PME associated with infected tissue is of host origin, and that its release has been accelerated by some host-pathogen interaction. Source of PG appears to be host as well as pathogen, because it has been detected in healthy tissue extract, as well as in culture medium. Other enzymes associated with infected tissue (PMG, PGTE, PMTE and Cellulase) appear to be of pathogen origin. These enzemes are secreted by the pathogen in culture medium, and they were not detected in the extracts of healthy tissue.

Plant tissues contain pectic material in middle lamella, as well as impregnated in the cellulose skeleton of the cell walls. While cellulose skeleton of the cell is degraded by the cellulase, pectic material of the cell wall is degraded by pectic enzymes. This is necessary for the spread of the pathogen in host tissue. Soft rot symptoms like maceration of tissue, water oozing and concavity in the infected area are the results of the action of enzymes on host tissue. These enzymes thus work for the initial parasitic stage of the pathogen, and subsequently for the saprophytic stage that follows, by making the degraded material available to the pathogen.

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1. Van Etten, H. D., Maxwell, D. P. and Bateman, D. F., *Phytopathology*, 1967, 57 (2), 121.
2. Kertesz, Z. I., *The Pectic Substances*, Inter Sc. Pub., New York, 1951, p. 628.

VARIABILITY IN *XANTHOMONAS VESICATORIA* (DOIDGE) DOWSON FROM TOMATO, CHILLI AND DATURA

Pathogenic variability in *Xanthomonas vesicatoria* was reported early in 1941 by Burkholder and Li[1]. Since then many workers have attempted to study the variability in this bactrium with varying results; (Dye *et al.*[3], 1964; Cook and Stall[2], 1969). Studies were conducted with eight isolates, three from tomato, four from chilli and one from datura. Morphologically all the isolates from tomato and chilli were alike in characters such as motility gram-reaction and flagellation. They behaved alike in most of the biochemical and physiological properties such as hydrolysis of starch, sodium hippurate, etc. In the utilisation of carbon compounds (sugars) and production of acid, the chilli isolates, gave delayed and mild acid reaction in celloboise galactose dextrin and melliboise, whereas lactose and mannitol were utilised only by the tomato isolates. In disc-gel electrophoretic comparison of the soluble protein patterns, 12 bands were commen in all, tomato and chilli isolates exhibited identical patterns, having 16 bands compared to 22 in datura.

Bacteriophages, isolated from chilli and datura were very specific to their homologous bacterial isolates. The chilli phage did not attack 20 other xanthomanads and 2 seudomonads tested but the datura phage typed *X. sesami* and *X. corcori*. In pathogenicity trials and cross inoculation experiments, it has been observed that the tomato isolate is not virulent on chilli, the chilli isolate readily infects both chilli and tomato, the tomato and chilli isolates could infect datura while the datura isolate could not infect chilli or tomato.

Based on the evidence obtained in the studies on physiological characters, protein, bacteriophage sensitivity and pathogenicity of tomato, chilli and datura isolates of *X. vesicatoria*, it is considered that they represent three different strains of the pathogen and that the tomato and datura strains might have evolved from the chilli strain. Further, the original host of the pathogen might have been chilli as evident by its ability to infect both tomato and datura. Moreover, the disease was recorded for the first time on chilli (Gardener and Kendrick[4], 1921). The tomato isolate was less specialised in that it could infect datura whereas the datura isolate was more specialised and could not infect tomato or chilli.

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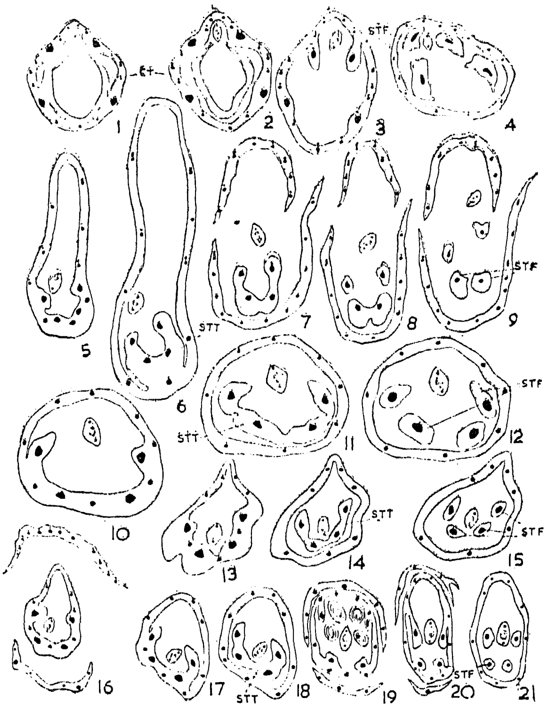
1. Burkholder, W. H. and Li, C. C., *Phytopathology*, 1941, 31, 753.
2. Cook, A. A. and Stall, R. E., *Pl. Dis. Reptr.*, 1969, 53, 617.
3. Dye, D. W., Starr, M. P. and Stolp, H., *Phytopathol. Z.*, 1964, 51, 394.
4. Gardener, M. W. and Kendrick, J. B., *Purdue Agric. Expt. Sta. Bull.*, 1921, 251, 1.

A NOTE ON MONADELPHY IN SOME LAMIACEAE (LABIATAE)

Epipetalous free stamens are reported in all the genera of Lamiaceae by Hooker (1885), Mukherjee (1940), Rendle (1959), Hutchinson (1959), Lawrence (1965) and Mitra (1965) except in *Coleus* where they are epipetalous and monadelphous. During a comparative floral anatomical studies on some taxa of Indian Lamiaceae, the author observed some interesting morphological features of the androecium which are recorded here.

It is observed in the present investigation that in *Anisochilus polystachyus* (Figs. 16–18) and *A. carnosus* (Figs. 13–15) the filaments of the stamens fuse at their base to form an incomplete tube. It extends to a height of 100–150 μ after the stamens get separated from the corolla tube. A similar condition has also been noticed in *Geniosporum prostratum* (Figs. 10–12) but the tube is comparatively shorter (50–100 μ) than the two species of *Anisochilus* investigated. However, in another species of *Geniosporum* (*G. indicum*) the staminal filaments are quite free from the point of their origin (Figs. 19μ21). Of the two species of *Coleus*, namely, *C. blumi and C. scutellarioides*, only the former shows monadelphous condition (Figs. 5–9) while the latter manifests a nonvascular tubular outgrowth developing in between corolla and androecium and the staminal filaments are free as in *Geniosporum indicum*. The coronary tube recorded in *C. scutellarioides* extends upwards and terminates below the place of emergence of stamens (Figs. 1–4). Thus from the present study, it is obvious that the union of the staminal filaments (monadelphy) is not confined to *Coleus* alone, but it also occurs in two more genera, namely, *Anisochilus* and *Geniosporum* though there is a marked variation in the extent of its development in the latter representatives. Monadelphous androecium, which represents a highly evolved condition (Bassey, 1915; Hutchinson, 1959), is not known in other families of Tubiflorae of Rendle (1959). In view of this observation it may now be considered that, this feature, may furnish additional support to the view that the family is more evolved among the Tubiflorae.



FIGS. 1–21. Serial transverse sections of flower buds of *Coleus*, *Geniosporum*, and *Anisochilus*. Figs. 1–4. *Coleus seutellariodies* × 20; Figs. 5–9. *Coleus blumii* × 20; Figs. 10–12. *Geniosporum prostratum* × 40; Figs. 13–15. *Anisochilus carnaosus* × 35; Figs. 16–18. *A. Polystachyus* × 35; Figs. 19–21. *Geniosporum indicum* 19 × 32, × 25 (CT–Coronary tube; STF–Staminal filaments, STT–Staminal tube).

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---

1. Hooker, D., "Labitatae," In Bentham and Hooker, *Genera Plantarum*, London, 1885.
2. Bessey, C. E., "The phylogenetic taxonomy of flowering plants," *Ann. Mis. Bot. Gdn.*, 1915, 2, 109.
3. Hutchinson, J., *Families of Flowering Plants*, 1969, Vol. II.
4. Lawrence, G. H. M., *Taxonomy of Flowering Plants.*, MacMillan Co., New York, 1965.
5. Mitra, J. N., *An Introduction to Systematic Botany and Ecology*, Calcutta, 1964.
6. Mukherjee, S. K., "A revision of the labitae of the Indian empire," *Rec. Bot. Sur. India*, 1940, p. 14.
7. Rendle, A. B., *The Classification of Flowering Plants*, Vol. II. *Dicotyledons*, Cambridge, England, 1959.

SOLHEIMIA KAMATII SP. NOV. (HYPHOMYCETE) A NEW GENERIC RECORD TO INDIA

RECENTLY, the senior author (KIM) collected a hyphomycetous fungus on the dead leaves of *Pandanus* sp. at Wynaad, N. Kerala, which agreed in all respects with a species of the form genus, *Solheimia* Morris. This form genus was established by Morris (1967)[1] for a monotypic phragmosporous, phaeostilbaceous member of Moniliales, with *S. costaspora* Morris as type, originally described from Panama Canal.

On critical study and comparison the collection proved to be significantly distinct from the type species, *viz.*, *Solheimia costaspora* Morris in possessing much longer and double dichotomously branched conidiophores with smaller conidia. The present collection has therefore been identified as a new species, *Solheimia kamatii* sp. nov. (Fig. I).

Synnemata errecta, cylindracia, viridascentia vel viridonigra, usque ad 1 mm altitudine, usque ad 25–60 μ latitudine; conidiophora cylindrica, errecta, pallidae vel fusco-virides, verrucosae, 2–2·5 μ latitudine, aggregata, non-ramosa ad basin, ramosa et hyalina ad apicem; conidia acrogenis, solitaria, aseptata, cum 6–8 costis longitudinalibus, fusiformata, olivacea vel fuscoviridia, magnit 10–12 × 2·5–3·5 μ, mucosa.

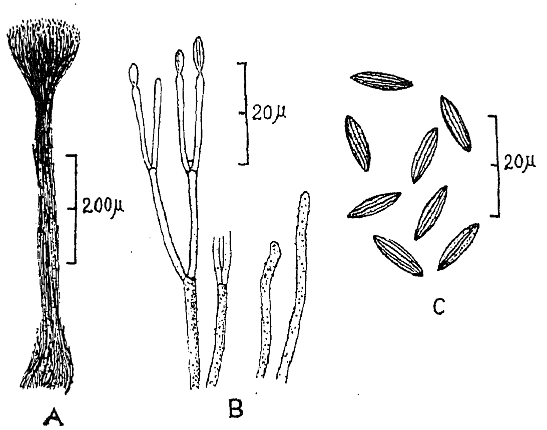

FIG. 1. *Solheimia kamatii* sp. nov. A. Synnemata; B. Conidiaphores with conidia; C. Conidia.

Matrix: On dead leaves of *Pandanus* sp. (F. Pandanaceae) Legit. K.I.M.V. (20-1-1976) at Wynaad, Kerala, No. AMH 2869 (Holotypus).

The genus *Solheimia* reported here constitutes a new generic record and an addition to the Fungi of India[2]. The specific ephithet has been chosen in honour of Prof. M. N. Kamat for his well-known contributions to Indian Mycology and Plant Pathology.

The writers are grateful to Prof. M. N. Kamat for his keen interest, to the Director for laboratory facilities, and to the Ministry of Education, Govt. of India, for the award of S.R.T. to one of them (K. I. M. V.).

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Plant Pathology, V. G. RAO.
M.A.C.S. Research Institute,
Poona 411 004 (India),
May 14, 1976.

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1. Morris, E. F., *Mycopath. et Mycol. Appl.*, 1967, 33, 181.
2. Mukerji, K. G. and Juneja, R. C., *Fungi of India,* Emkay Publ., Delhi, 1975, p. 224.

CARPELLARY VASCULATURE AND THE OVULAR VASCULAR SUPPLY IN *UVARIOPSIS GLOBIFLORA* L. AND *MONOCYCLANTHUS VIGNEI* KEAY

VASCULARIZATION of the ovules by the carpellary dorsal bundle in two species of the Annonaceae, *e.g.*, *Cananga odorata* H. f. and T. and *Saccopetalum tomentosum* H. f. and T., has been described. During the course of a study of the floral anatomy of the family, the present author has observed the same feature in two African species. Since such a type of vascularization is a rare feature, it may be worthwhile to put it on record.

The carpel in both the present plants, *Uvariopsis globiflora* L. and *Monocyclanthus vignei* Keay, is open for a greater part of its length with only the margins appressed closely. In *U. globiflora,* the ovules are many in number and are two-seriate in a carpel. They arise a little distance away from the margin so that the placentation appears laminar. *M. vignei* shares these features, but the number of ovules is fewer, generally 6–8 per carpel.

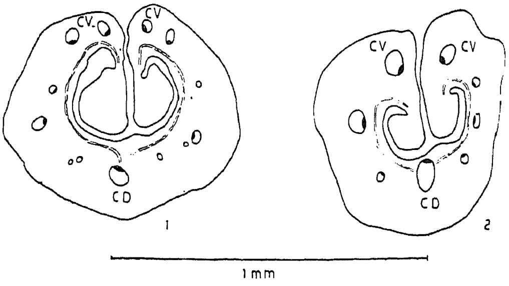

FIG. 1–2. Fig. 1. *Uvariopsis globiflora.* Fig. 2. *Monocyclanthus vignei.* CD, Carpellary dorsal. CV, Carpellary ventral.

In both these species, the carpels have five principal traces—a carpellary dorsal, two carpellary ventrals and two laterals. The latter generally bear a few lateral branches which establish as tiny strands in the carpellary wall, while each of the carpellary ventral bundles bifurcates sooner or later into two strands. The carpellary dorsal bundle bears branches on either side which travel in the carpellary wall towards the ventral side and then enter the ovules. These traces are procambial even in the mature carpel[4].

Vascularization of the ovules by the carpellary dorsal bundle is a relatively primitive feature observed only in some of the ranalian taxa[1-6]. In the Winteraceae and the Degeneriaceae, where the carpels show a number of primitive features, the ovules receive their vascular supply from both the carpellary dorsal and carpellary ventral bundles. The present observations are significant because the dorsal bundle alone is concerned with the vascular supply to the ovules in these annonaceous plants which otherwise show a number of advanced characters and also because this feature is of more common occurrence within the family as observed by Sastri[5]. They may also be taken as additional evidence in support of the conduplicate nature of the carpel.

The author is grateful to Dr. R. M. Pai for his guidance and comments, and Prof. K. B. Deshpande for facilities. She thanks Mr. A. A. Enti, Ghana for the material of the two species studied.

Plant Morphology (MISS) MEENAKSHI LYAGALWAR
Laboratory,
Botany Department,
Marathwada University,
Aurangabad, *May* 8, 1976.

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1. Bailey, I. W. and Swamy, B. G. L., *Amer. J. Bot.*, 1951, 38, 373.
2. Canright, J. E., *Ibid.*, 1960, 47, 145.
3. Esau, K., *Plant Anotomy*, 2nd ed., John Wiley and Sons, 1965.
4. Periasmy, K. and Swamy, B. G. L., *J. Arnold Arb.*, 1956, 37, 366.
5. Sastri, R. L. N., *Curr. Sci.*, 1957, 26, 183.
6. —, *Biol. Rev.*, 1969, 44, 291.

PETRIFIED SPOROCARPS OF SALVINIACEAE

MEGASPORES of *Salvinia* were first recorded from the Intertrappean Cherts of Sausar by Sahni and Srivastav (1934) under the name *Sausarosparmum fermori* thinking them to be seeds of Gymnosperms. These were later referred to the family Salviniaceae by Mahabale (1950) as megaspores of *Salvinia intertrappea.* No sporocarps, however, were described by either of the authors.

Present specimen from a piece of Deccan Intertrappean Chert of Mohgaonkalan, which is about 60 miles away from Sausar, shows the same type of megaspore, as described earlier by above authors, inside a distinct sporocarp (Fig. 1). Preservation of this spore and the sporocarp is good. The latter contains remnants of some decayed tissue, possibly the aborted spores. Sporangial wall is not preserved except for a few traces.

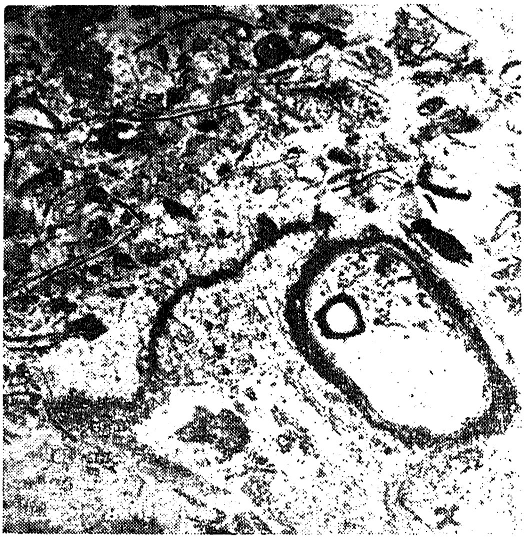

FIG. 1, × 4·5 (*x*—Second sporocarp).

As is seen in living *Salvinia* the wall of the sporocarp in this fossil specimen is formed by a modified leaf. Sporocarps are developed here in a row, since a little portion of a second sporocarp (Fig. 1 *x*) is seen adhered at the distal end of the first complete sporocarp.

Along with these sporocarps in close association many roots (modified leaves), stems and complete leaves, looking very much like those of *Salvinia,* are nicely preserved. These will be described at a later date.

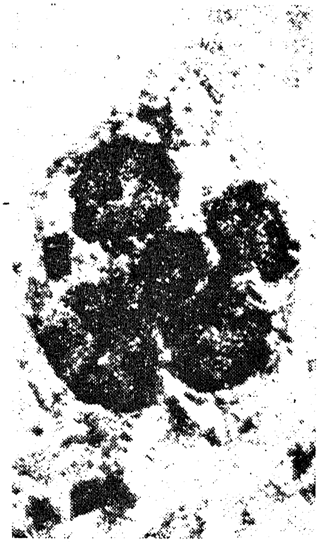

FIG. 2. *Azolla microsporocarp,* × 50.

In the same specimen a developing young microsporocarp is observed (Fig. 2) with an indusium, enclosing microsporangia with massulae and spores. Glochidia are not fully developed. This microsporocarp, no doubt, belongs to *Azolla,* probably *A. intertrappea.* So far such a complete microsporocarp with indusial wall has not been described by either Sahni (1941) or Rao (1957). The latter gave a report of few groups of sporangia together, with a suggestion that they were surrounded by a sporocarp wall, traces of which were observed by him. However, neither photos given by him show any clear sporocarp wall, nor it is described by him in detail. Hence the present report will be the first record of a complete young developing microsporocarp of *Azolla* from these beds.

This discovery of these petrified fertile parts of Salviniaceae from the Deccan Intertrappean beds of Mohgaonkalan is of great significance. Detailed description of both the sporocarps—Megasporocarps of *Salvinia* and microsporocarp of *Azolla*—along with the vegetative parts of the former, will be published after further investigation.

Department of Botany, S. D. CHITALEY.
Institute of Science,
Bombay 400 032, *March* 1, 1976.

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1. Mahabale, T. S., *Nature*, 1950, 165, 410.
2. Rao, A. R., *The Palaeobotanist*, 1957, 6, 19.
3. Sahni, B., *Proc. Indian Acad. Sci.*, 1941, 14, 489.
4. — and Srivastav, B. P., *Proc. 21st Indian Sci. Congr.*, 1934, p. 318.

SHORT SCIENTIFIC NOTES

A Carangid Fish *Caranx williamsi* (Smith, 1968) (Pisces: Carangidae)

A survey of the carangid fishes of Porto Novo coast showed 24 species to occur here, and in addition a new carangid fish *Caranx williamsi*[1] hitherto unreported from Indian waters has also been recorded. Earlier reports indicate the distribution of the species is restricted to South Africa, Kenya and Western Indian Ocean[1].

Ten fishes ranging from 96 mm to 113 mm in standard length were obtained from the commercial catches on 20–8–1976. All of them were found to be juveniles.

All these specimens fit the original description given by Smith (1968), except in eye diameter 2·9 cf 4·1–4·3 in head and number of Scutes in the lateral line 34–38 cf 35.

According to Sreenivasan (per. comm.) this species has been noticed in the west coast also and this confirms the occurrence of this species in the Indian waters around the peninsular region.

We are indebted to Prof. R. Natarajan, Director, for guidance. One of us (V. K. V.) is also thankful to the U.G.C., New Delhi, for the award of a Junior Research Fellowship.

Centre of Advanced Study
in Marine Biology,
Annamalai University,
Parangipettai 608 502,
Tamil Nadu, *October* 8, 1976.

V. K. Venkataramani•
V. Ramaiyan.

---

1. Smith, J. L. B., *Rhodes Univ. Dept. Ichthyol. Occ Pap.* (No. 15), 1968, p. 173.

Leaf Crumpling New Syndrome in Citrus

During 1967, Mosambi tree on rough lemon stock at Ganeshkhind Fruit Experimental Station, Pune, was found to have diagnostic leaf symptoms, *viz.*, crumpling, cupping, puckering and abnormal leaves. Preliminary experiments revealed that the above syndrome on Mosambi leaves is viral in nature and is named as "Leaf crumpling virus of Mosambi".

Transmission and host range studies indicated that the syndrome was graft and bud transmitted but not transmitted by sap nor by *Toxoptera citricidus* Kirk. and *Diaphorina citri* Kuway. Host range was restricted to 6 *Citrus* spp., *viz.*, *C. reticulata*; *C. sinensis*; *C. aurantifolia*; *C. paradisi*; *C. limonia* and *C. jambheri*. Thus the above studies pointed out that syndrome is not associated with greening disease but appears to be a new Mosambi virus disease having new diagnostic leaf syndrome.

Plant Virus Research Station,
Agricultural College Estate,
Pune 411 005,
November 19, 1976.

T. S. Marathe.*
P. K. Pandey.

---

* Present address : Junior Plant Pathologist, Citrus Die Back Scheme, Punjabrao Krishi Vidyapeeth, Akola 444 004.

Powdery Mildew of *Nicandra physaloides* Gaertn.– A New Record

During the course of a general survey of potato diseases, a hitherto unrecorded powdery mildew of *Nicandra physaloides* Gaertn. showing the characteristic disease symptoms on leaves, stems and berries was observed in the Nilgiris during the month of December 1975. Microscopic observations of the infected leaves showed the presence of an *Oidium* species, having a creeping, hyaline, septate and branched superficial mycelial mats with 4·5 to 5·4 μ in diameter and forming a sparse to thick coating on both the surfaces o the leaf lamina. In the absence of any cleistothecial bodies, the exact species of the pathogen, however, could not be ascertained.

The conidiophores of the pathogen are simple, erect and usually 2 to 3 septate and measure 40·5–58·5 × 4·5–11·25 μ (51·25 × 8·25 μ) in length and width respectively and bear a chain of three to four amerosporous, hyaline, ovoid to elongate, sometimes zig-zag sculpturing on exine with smooth entine walled conidia produced in a basipetal manner, measuring 22·5–31·5 × 13·5–18·0 μ (27·5 × 16·5 μ) in length and width respectively.

In literature, there is no record of any *Oidium* sp. on *N. physaloides* and thus this is the first record from India or elsewhere.

The authors wish to express their deep sense of gratitude to Dr. A. Johnston and Dr. A. Sivanesan, Director and Mycologist respectively of the Commonwealth Mycological Institute, Kew, Surrey, U.K., for kindly confirming the identity of the pathogen and deposition of the diseased specimen in their herbarium (IMI-201956).

Department of Plant Pathology,
Central Potato Research Institute,
Simla (H.P.), *October* 11, 1976.

D. S. Singh.
B. B. Nagaich.

Additional Host of Tristeza Virus Disease

Tristeza virus has a wide host range in India (Capoor, 1963). During host range studies of the virus, the budded 2-year old seedlings of *Citrus macroptera* Montrousier produced diagnostic symptoms of marginal yellowing after 6 months, which were masked at high temperatures. Infected seedlings had normal growth with a number of branches. Presence of stem pitting was also observed. Indexing on Kagzi lime seedlings produced characteristic Tristeza virus reaction, thus confirming that *C. macroptera* is susceptible to Tristeza virus and incidentally this has been experimentally demonstrated for the first time in India. These findings agree with those of Grant *et al.* (1950), Bitters *et al.* (1951) and Knorr (1957). It may be indicated that *C. macroptera* is an additional host of Tristeza virus in India.

Plant Virus Research Station, T. S. MARATHE*.
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Pune 411 005, *November* 19, 1976.

---

* Present address : Junior Plant Pathologist, Citrus Die Back Scheme, Punjabrao Krishi Vidyapeeth, Akola 444 004.

1. Bitters, W. P. and Parker, E. R., *California Citrograph*, 1951, **36**, 313, 329.
2. Capoor, S. P., *Nat. Inst. Sci. India Bull.*, 1963, **24**, 48.
3. Grant, T. J., Costa, A. S. and Moreira, S., *Proc. Florida State Hort. Soc.*, 1950, **62**, 72.
4. Knorr, L. C., *Proc. of the Conference of Citrus Virus Diseases*, 1957, p. 63.

Two New Fungal Leaf Spot Diseases

During months of September and October 1975 severe leaf spot diseases of *Cordia dichotoma* and *Strelitzia reginae* were observed in the Saugar University Botanical garden. The pathogens were isolated from the affected parts by pathological techniques on potato dextrose agar. The microscopic examination of the cultures showed the presence of *Gloeosporium fructigenum* Berk on *C. dichotoma* and *Alternaria alternata* (Fr) Keissler on *S. reginae*. The pathogeniety was established on detatched leaves in both the cases.

In case of *C. dichotoma*, the disease appears after rainy season in the form of numerous brown necrotic spots, 2–4 mm in diameter, scattered all over the leaf surface. The spots later enlarge, sometimes fuse with each other, and turn dark brown in colour with black outline. Acervuli develop on the spots later on. In laboratory typical symptoms developed after 3–4 days of inoculation.

In *S. reginae*, infection starts at the margin of leaf and proceeds towards the midrib. The diseased parts become grey in colour later changing to brown. The affected leaves then shrivel up and dry out. In laboratory, the typical symptoms appeared after 5–6 days of inoculation.

Both the isolates resembled with the type descriptions except minor variations in the size of conidia. A perusal of literature revealed that the above fungi are not reported from the respective hosts so far. The living cultures have been deposited at I.T.C.C., Indian Agricultural Research Institute, New Delhi, under accession number 2078 (*G. fructigenum*) and 2077 (*A. alternata*).

The authors are grateful to U.G.C. for providing financial assistance to one of us (T. S. T.).

Department of Botany, T. S. THIND.
Saugar University, S. B. SAKSENA.
Sagar (M.P.) 470 003, S. C. AGRAWAL.
May 3, 1976.

New Diseases of Sugarcane in U.P., India

A new leaf spot disease has been observed on sugarcane varieties Co. 1148, Co. 1158 and B.O·17 during 1974–75 in Pilibhit, Muzaffarnagar and Lakhimpur Districts of U.P. (India). The disease appeared early in August with the onset of rains. All the leaves except 4–5 spindle leaves are affected. The spots are rounded or oval and scattered all over the blade o the leaf. The size of the spots vary from a pinpoint to 1·5 and are red in colour.

The pathogen responsible for the madady was identified as *Hyalostachybotrys sacchari* Srinivasan.[1] The fungus grows well in Oatmeal agar medium (OMA) at 25° to 28° C. Preliminary investigation on its effect on juice quality revealed more invert sugar in diseased plants. Appearance of a 'Leaf scald' like disease, of bacterial, origin was also observed on a promising variety BO·70 at Sugarcane Research Farm, Gorakhpur, during January 1976. These are the first incidences of the diseases and have not been reported earlier from U.P.

Sugarcane Research Station, S. C. GUPTA,
Shahjahanpur (U.P.),
August 10, 1976.

---

1. Srinivasan, K. V., *J. Indian. bot. Soc.*, 1958, **37** 340.

Note on Two Fungi from Sugarcane Leaf

Two fungi, *viz.*, *Chaetomium globosum* Kunze, ex. Fr. (IMI. 185291) and *Nigrospora sphaerica* (Sacc.) Mason (IMI. 185290) found growing on all parts of dead and drying sugarcane plants were isolated. *Chaetomium globosum* Kunze, ex. Fr. is the commonest species isolated from seeds of several cultivated crops and forest trees (Skolko and Groves[3]) but not from the leaves of sugarcane while *Nigrospora sphaerica* (Sacc.) Mason is reported from sugarcane leaf by Hudson[1] from West Indies, Patouillard[2] from

S. America and Sydow[4] from Philippines. It appears to be first record of these fungi from sugarcane in India.

Thanks are due to Dr. Ellis and Dr. Hawksworth of C.M.I., Kew, for identification of the fungi.

Sugarcane Research Station
Padegaon, District Poona,
November 22, 1976.

M. B. Bachchhav.

---

1. Hudson, H. J., *Trans. Brit. Mycol. Soc.*, 1962, **45**, 395.
2. Patouillard, N. and De Lagerheim, G., *Bull. Soc. Myc. Fr.*, 1893, **9** (2 and 3), 124.
3. Skolko, A. J. and Groves. J. W., *Canad. J. Bot.* 1953, **31**, 779.
4. Sydow, H. and Annal, P., *Mycol.*, 1913, **11** (3), 254.

A New Variety of *Achlya stellata* de Bary

Achlya stellata var. *multispora* var. nov.

A typo differt hyphae tenuer, oosperae 3–5.

Mycelium moderately extensive, diffuse, hyphae slender, branched, measuring 22·16–66·66 μm in diameter at the base. Zoosporangia moderately abundant, fusiform or clavate, 155·54–255·53 μm × 22·22–44·44 μm, renewed sympodially. Zoospores discharge achlyoid, encysted zoospores 5·54–8·31 μm in d meter. Gemmae absent. Oogonia abundant lateral branches, globose measuring 44·44–88·64 in diameter inclusive of ornamentations. Oogo wall unpitted. Antheridia generally present, mo clinous, laterally appressed to the oogonium. Oospher commonly 3–5 in an oogonium, spherical filling oogonium. Oospheres subcentric, 19·39–27·70 in diameter.

Isolated in March, 1976 from alkaline muddy s (pH·8·0) collected from Utrethiya fisheries fa Lucknow.

This new variety differs from already describ isolates of *A. stellata*[1, 2] in having typical and regu presence of 3–5 oospheres per oogonium, relative thinner hyphae and smaller zoosporangia.

Department of Botany,
Lucknow University,
Lucknow–226007, *August*, 27, 1976.

J. N. Rai.
J. K. Misra.

---

1. Dick, M. W., *Trans. Brit. Mycol. Soc.*, 196 **43** (3), 479.
2. Srivastava, G. C., *Curr. Sci.*, 1975, **44** (4), 139

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REVIEWS AND NOTICES OF BOOKS

General aad Applied Entomology. By K. K. Nayar, T. N. Ananthakrishnan and B. Vasantharaj David. (Tata McGraw-Hill Publication Co. Ltd., New Delhi), 1976. Pp. 589. Price Rs. 96·00.

Entomological studies have made rapid strides over the last quarter of a century. There is hardly any text-book from India covering modern trends relating to structure and function and recent disciplines such as reproductive biology, neuroendocrinology, behavioural physiology, population ecology, toxicology and interdisciplinary approach to insect control of Indian pest species. This book written by three eminent entomologists of this country, specialists in their respective fields of entomological research, will serve as a useful contribution in fulfilling the gap that has been felt in our country. Late Dr. Nayar was one of our finest entomologists and a pioneer in insect endocrinology in India. Dr. Ananthakrishnan has been interested in diverse aspects of entomology and has specialised in Thysanoptera. Dr. David experienced in teaching, research and extension in the field of agricultural entomology has also specialized in Aleyrodidae.

This book comprises of three main sections, *viz.*, (i) Morphology, physiology, ethology and ecology, (ii) Insect orders, and (iii) Applied Entomology.

The first section on morphology, physiology, ethology and ecology comprises of 30 chapters. Apart from dealing with external and internal morphology in the traditional way special chapters incorporating modern trends have been included on topics such as nutrition in insects, intermediary metabolism, fat body, water and salt regulation, insect movements, senory receptors, endocrine system, sonification, rhythms, diapause, biology of reproduction, communication in insects, environment of insects, dynamics of insect population, social life of insects, taxonomy and fossils. In the chapter on taxonomy the phylogenetic tree showing inter-relationship of the insect orders is very well illustrated. The impact of biochemistry and physiology on the various aspects discussed in the section is quite evident.

The second section, Insect Orders, covers 29 orders of class Insecta which attempt in providing as complete a modern classification as possible. A new trend has been introduced laying emphasis on the bionomics and control of almost every pest species from India and to a limited extent Asian insects under each family. This approach has served to blend the pure and applied aspects. The host range of each species whether phytophagous, predatory or parasitic, is another useful information available for most insects. These also cover elaborately pests of crops, stored products, forest trees and animals, disease carriers in plants, animals and human beings, etc.

The third section, Applied Entomology, deals with toxicology relating to formulations, classification, chemical structure, mode of action and various compounds of pesticides. Special mention should be made of the chapters on systemic insecticides, hormone mimics as pesticides, low volume concentrates, aircraft application of pesticides, insecticide residues, median lethal dosage, resistance to insecticides, insect and host plants and insects and animal hosts. Aspects of control such as biological, microbial, genetic, behavioural and integrated methods of control have also been discussed. The chart on compatibility of spray chemicals is a very useful reference-sheet.

A further highlight of this book is the inclusion of a comprehensive list of insects infesting shrubs, plants and trees (including storage pests) in the country with common and scientific names, family and order. This would serve as an extremely valuable ready reference to students and research workers alike.

This book is profusely illustrated with line drawings, photographs and electron micrographs. A bibliography of pertinent references, a species index and a general index have also been provided.

Through a few printing mistakes are noticeable they can be avoided while taking up the second edition. However, the get-up of the book is attractive and considering the wealth of information it offers on various aspects of general and applied entomology the price is reasonable. The authors are to be congratulated on their valuable contribution to the cause of entomological research and education in the country.

V. G. PRASAD.

Annual Review of Plant Physiology (Vol. 27). (Annual Reviews, Inc., Palo Alto, California, 94306), 1976. Pp. 581. Price U.S.A. $ 17 :00; Elsewhere: $ 17.50.

In this volume of *Annual Review of Plant Physiology* in the field of Cell Organisation, three papers (1) Biosynthesis, Intracellular transport and Secretion of extracellular macromolecules by M. J. Chrispeels, (2) Nuclear organisation by W. Nagl, (3) Self-assembly in development by G. B. Bouck and B. L. Brown are published. In the field of Bioenergetics (1) The organisation and regulation of electron transport in plant mitochondria by J. M. Palmar, (2) Regulatory aspects of photosynthetic carbon metabolism by G. J. Kelly,

E. Latzko and M. Gibbs, (3) Paramagnetic intermediates in photosynthesis by J. R. Bolten and J. T. Warden, are covered. Under Metabolism the topics (1) Mobilisation of storage proteins of seeds by F. M. Ashton, (2) Plant RNA polymerases by C. T. Duda, (3) Post-translational modification of proteins by phosphorylation by A. Trewavas are reviewed. Under Development seven topics (1) The development of fleshy fruits by B. G. Coombe, (2) Biochemical and molecular genetic aspects of cellular slime mold development by M. Sussman and R. Brackenbury, (3) Hormone binding in plant by H. Kende and G. Gardner. (4) Physiology of flower formation by Jan A. D. Zeevaart, (5) Geotropism by B. E. Juniper, (6) Modelling of plant growth by R. O. Erickson, (7) Root hormones and plant growth by J. C. Torrey are reviewed.

Osmoregulation by J. A. Hellebust and Freezing and Injury in plants by M. J. Burke, L. V. Gusta, H. A. Quamme, C. J. Weiser, and P. H. Lee—have been reviewed under Environmental physiology.

Biological rhythms and physiological timing by W. S. Hillman, Phytohemagglutinins (Phytolectins by I. E. Liener, Physiological respondents of phytoplankton and major environmental factors by Olga V. H, Owens and W. E. Esaias are the special topics reviewed in this issue.

In the prefatory chapter, plant physiology and human ecosystem by J. Van Overbeek, the problem of world food supply in relation to human population has been highlighted. The need for inter-disciplinary approach and understanding of the basic physiological aspects to produce new varieties or new species with better productivity has been stressed. The statement "Plant Physiology is to Agronomy what human physiology is to medicine" is quite apt and signifies the importance of plant physiology in increasing the productivity.

K. S. KRISHNA SASTRY.

The Indian Ephemeris and Nautical Almanac for the Year 1977. Prepared by Nautical Almanac Unit. Regional Meteorological Centre, Alipore, Calcutta 700 027. (The Controller of Publications. Civil Lines, Delhi 110 054), Pp. xxii + 473. Price Rs. 30·00, Foreign : £ 3·50 or $ 10·80 c.

A very useful publication for all observing astronomers, *The Indian Ephemeris aud Nautical Almanac* 1977, is not much different from the earlier publications of the series. In the context of inevitable delays and difficulties in the fields of publishing scientific literature, the Director-General of Observatories and his staff do deserve congratulations for bringing out this volume in time.

A few observations, however, appear necessry f inviting the attention of the concerned authoriti The quality of paper and printing is far from des rable and innumerable small printing mistakes coul have been avoided with due care and precaution. Th size of the book has suddenly increased with hardl any increase in the volume of contents or more conv nient form of tabulation. The increase in size is like to create some difficulty in Libraries, where the succe sive annual volumes will be stored in future.

On the matter of form of tabulation, we can ci the example of pages 52–143 where hourly positio of the moon are tabulated. Twenty-four hourl positions are tabulated in a vertical column; o would welcome a space after say, every six lines, makin the reading less confusing. The columns could ha been better aligned, thereby greatly improving th look of this volume.

Since these data are obtained from the Britis Nautical Almanac Office, it is not clear why the pos tions are given with the last figure rounded off to higher decimal place. In the context of modern obs vations involving high precision timing, this is a maj handicap. Hourly values of the horizontal parall as given in the American Ephemeris would have bee useful in these calculations.

Similar shortcomings are noticed in other plac also, when one compares the publication with th other ephemeries published outside the country. I s hoped that these may be gradually removed in future volumes.

J. C. BHATTACHARYYA.

Elementary Differential Equations with Applications By W. R. Derrick and S. I. Grossman. (Addison Wesley Pub. Co., Inc., Reading, Massachusetts 01867) 1976. Pp. xv + 555. Price $ 13.50.

A study of differential equations is practically essential both in the case of Physical and Biological Sciences. The book under review contains 12 chapters. In the first three chapters the authors discuss the metho of finding solutions in elementary cases, mostly dealin with differential equations with constant coefficients. The development of difference equations alongside with differential equation is particularly useful. In recent years the difference equations are receiving attention since many types of equations can be solved numerically, using difference equation formulations employing the computer. There is a good development of difference equations in Chapter 2 Section 6 and also Chapter 4. Chapter 5 deals with the power series method of solving differential equations. After discussing the general methods of solving both in the case of differential equations with ordinary point and singular point, the authors have chosen the Bessel's differential equation and the Legendre differential equation for complete discussion. The Bessel functions and Legendre Polynomials are discussed in detail. The authors have left the treatment in the case of hermite differential equation and Laguerre differential equation as exercises to be worked out by the student. The application of Laplace transforms to the solution of ordinary differential equations and difference equations is dealt with, in Chapter 6. The non-s-multiplied transform is used as the definition. Chapter 7 deals with the treatment of systems of differential and difference equations. The discussion of systems from the point of view of matrix theory is very useful. Chapter 8 deals with numerical methods and the process by which a differential equation can be approximated by a difference equation is illustrated. It is a welcome feature that computer language (Fortran and Basic) is introduced to enable the students to be exposed to computer procedures at a fairly early stage in their study. Both homogeneous and non-homogeneous boundary value problems are dealt with in Chapter 9. The importance of Fourier series techniques in solving non-homogeneous boundary value problems is discussed along with a clear exposition of orthogonal sets of functions and Fourier series. In Chapter 10, a brief treatment of partial differential equations is given. Examples having important practical applications are discussed (*e.g.*, vibrating string, heat flow). The application of Laplace transforms for the solution of partial differential equations is discussed. Chapter 11 deals with the existence theorem. Since the differential equation is only a model of a physical problem, it is likely that if the model is bad, there may not be any solution at all. Inclusion of the existence of uniqueness theorems is of great value since it can tell us the equations that have solutions. Chapter 12 deals with a discussion of Lyapunov stability theory for differential equations.

There are four appendices (integral tables, Laplace Transform Tables, Fortran-Basic Primer, determinants). Particularly the introduction to the Fortran-Basic computer languages presented in Appendix 3 is quite useful. Illustrations with reference to situations from Physical and Biological sciences is a welcome feature.

The reviewer has no hesitation in recommending this book to all students interested in the study of differential equations.

R. S. SUBRAHMANYA.

Controlled Climate and Plant Research. (Technical Bulletin No. 148 of WMO). By R. J. Downs and H. Hellmers. (WMO, Geneva, Switzerland), 1976, Pp. 60. Price: Not given.

Few contributions satisfy the twin criteria of 'timelines and purpose' as that of the Technical Note No 148 *Controlled Climate and Plant Research* by R. J. Downs and H. Hellmers brought out at the behest of World Meteorological Organisation, The authors have encompassed the whole range of scope and limitations of 'reach-in' cabinet of growth chamber as well as 'walk-in' rooms of phytotrons. Light, Temperature, Relative Humidity and Carbon Dioxide, as major controlling factors, have been examined with academic thoroughness. Similarly the versatility in phytotronics in special design has been amply dealt with. Despite the masterly treatment the authors appear to shy away from recommending at least a few limited types for specific studies at University level or commercial ventures. It is difficult not to expect suggestion to the readers of the note, though possibly the task assigned to the authors could have been specific. The utility of the knowledgeable note, by research workers in the developing countries, particulalry in the 'tropics' is very much limited The chapter on Controlled Environment is in fact an attempt, though feeble, to satisfy the need for information on 'simulation modelling', but one may expect more. It may be hoped that more notes on allied aspects are forthcoming under the auspices of WMO. It is felt that the authors could have presented information on certain topics such as 'Lighting Systems , Refrigeration Systems', 'Special Designs' in an abridged fashion in tabular statements that is bound to elicit appreciation for catching the eye of the reader and economy in space. The authors are to be congratulated for their unique success in the onerous taste.

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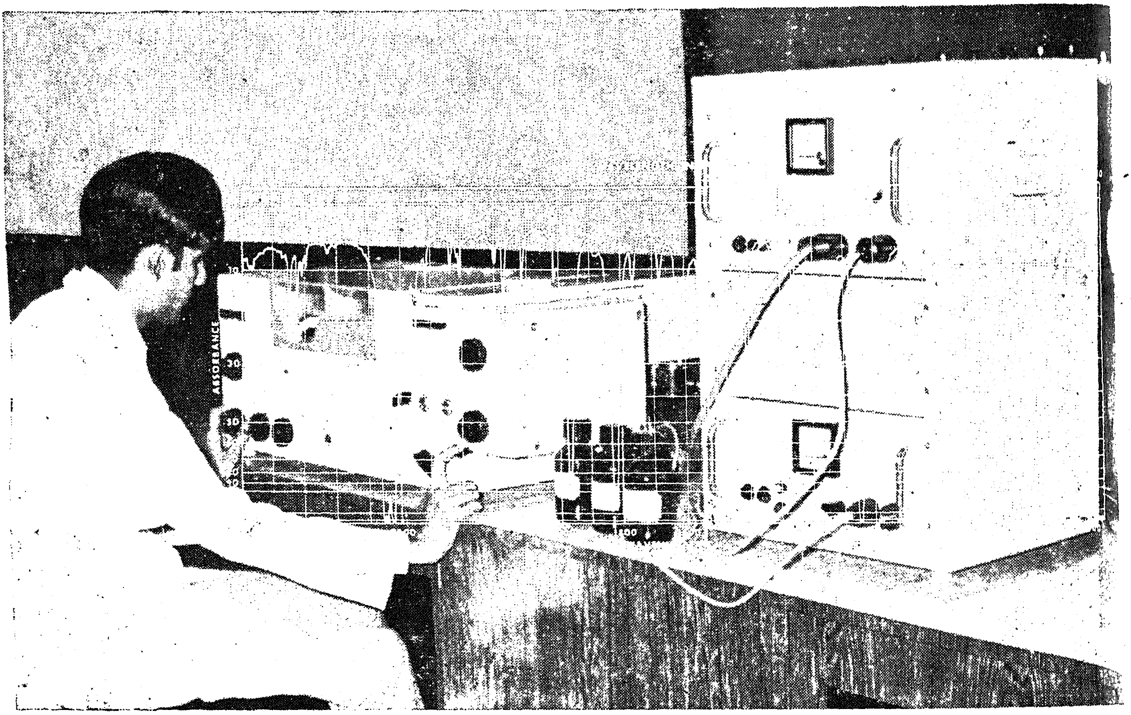

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THE RKRV CURVES AND DISSOCIATION ENERGY OF AlF MOLECULE

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ABSTRACT

The true potential energy curves for $X^1\Sigma^+$. $A^1\Pi$, $C^1\Sigma^+$, $D^1\Delta$, $E^1\Pi$, $F^1\Pi$ $b\,^3\Sigma^+$, $c\,^3\Sigma^+$ states of AlF molecule have been calculated by Lakshman and Rao's (present authors) method as well as Rydberg Klein-Rees as modified by Vanderslice *et al.* method, the agreement between the two is excellent. Dissociation energy and Ionization Potential for this molecule have been estimated as 7·016 eV and 9·696 eV respectively, which are in good agreement with the values given by Barrow *et al.*

INTRODUCTION

THE band spectrum of AlF has been investigated in emission by many investigators[1–5] and recently by Barrow *et al.*[6] and in all, twelve band systems for AlF molecule have been observed and analysed. The rotational and vibrational constants required in the present work are presented in Table I.

TABLE I

Molecular constants of AlF molecule

State	Te cm^{-1}	ω_e cm^{-1}	$\omega_e x_e$ cm^{-1}	$\omega_e y_e$ cm^{-1}	B_e cm^{-1}	$\alpha_e \times 10^3$ cm^{-1}	r_e Å
$X^1\Sigma^+$	0	802·26	4·77	..	0·55250	4·95	1·6542
$A^1\Pi$	43949·2	803·94	5·99	−0·050	0·55640	5·34	1·6484
$C^1\Sigma^+$	57688·0	938·22	5·09	−0·017	0·58992	4·58	1·6009
$D^1\Delta$	61229·5	901·05	6·11	..	0·58297	5·02	1·6104
$E^1\Pi$	63689·4	923·02	5·28	..	0·58709	4·64	1·6047
$F^1\Pi$	65795·6	955·33	5·38	..	0·59281	4·59	1·5970
$b\,^3\Sigma^+$	44813·2	786·37	7·64	−0·009	0·56280	6·51	1·6390
$c\,^3\Sigma^+$	54957·7	933·66	4·81	..	0·58861	4·57	1·6026

The molecules N_2, CO, BF, SiO, SiS, CS and AlF have rather similar spectra. In continuation of the work on SiO and SiS molecules, the authors took up the present work on AlF molecule.

The construction of potential energy curves is of considerable importance for the understanding of kinetic mechanisms, spectral phenomena, stellar structure and many associated problems[7–9]. Potential energy minima determine the possible structures and are thus related to bond strength and valence. The curvatures of the potential energy surface, the second derivatives of the potential energy with respect to the distance in certain specified directions are the force constants for various modes of vibration and determine the vibrational and rotational energy levels of the molecule. The positions of these vibrational and rotational energy levels determine the thermodynamic properties of the substances. Gas imperfections are due to potential energy changes in molecular collisions while reaction rates are determined by the potential energy surface of the activated complex of the reacting molecule.

THE TRUE POTENTIAL ENERGY CURVES

The present paper deals with the construction of potential energy curves for different electronic states $X^1\Sigma^+$, $A^1\Pi$, $C^1\Sigma^+$, $D^1\Delta$, $E^1\Pi$, $F^1\Pi$, $b\,^3\Sigma^+$, $c\,^3\Sigma^+$ of AlF molecule using the Rydberg-Klein-Rees method as modified by Vanderslice *et al.*[10–11] along with the Lakshman and Rao's method[12]. The RKRV method has been considered to be superior to the Morse method, since it can be used upto the dissociation limit, unlike Morse method whose validity lies in regions near the potential minimum. Even though the vibrational levels are few in number in the different states of AlF molecule, the RKRV method is preferred to the Morse method, since the α_e as calculated from Pekeris relation (Herzberg)[13] deviates ranging from 1 to 11% in different electronic states to the experimentally observed α_e values in the different electronic states. This method which is known as RKRV method is a WKB approximation from which we obtain reliable potential energy curves from the measured vibrational and rotational analyses of the diatomic molecules.

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The present method by Lakshman and Rao[12] was successfully verified by them in number of cases[14–16] and recently Chakraborty and Pan[17] in their review paper gave a detailed account of different methods and the authors' method is one such reliable and accurate, which involves less complicated mathematical calculations. Since the details of this method are reported in literature[12], only the explicit formulae to evaluate the classical turning points are given below:

$$f = \left[\frac{8\pi^2 \mu c\,(\omega x)_i}{h}\right]^{-1/2} \ln W_i$$

$$g = \left(\frac{2\pi^2 \mu c}{h}\right)^{1/2} \{2a_i\,(\omega x)_i^{-1}\,U_i^{1/2}$$

$$+ (\omega x)_i^{1/2}\,[2B_i - a_i\,\omega_i\,(\omega x)_i^{-1}] \ln W_i\}$$

where

$$W_i = \frac{\omega_i^2 - 4\,(\omega x)_i\,U_i}{\omega_i - 2\,(\omega x)_i^{1/2}\,U_i^{1/2}}$$

the symbols have their usual spectroscopic significance. The classical turning points are given by the relation

$$r_{\max,\ \min} = \left(\frac{f}{g} + f^2\right)^{1/2} \pm f.$$

RESULTS AND DISCUSSION

Employing the authors' method the potential energy curves for the different electronic states of AlF molecule have been calculated and the results are presented in the Table II. The agreement between the RKRV

TABLE II

The true potential energy curves for different electronic states of AlF molecule

		RKRV method		Authors' method	
V	U + T$_e$ (cm^{-1}) 1	$r_{\max}$ (Å) 2	$r_{\min}$ (Å) 3	$r_{\max}$ (Å) 4	$r_{\min}$ (Å) 5
	T$_e$ = 0		X$^1\Sigma^+$ state		
0	399·9	1·719	1·596	1·719	1·596
1	1192·7	1·772	1·558	1·772	1·558
2	1975·8	1·811	1·533	1·811	1·533
3	2749·5	1·845	1·514	1·845	1·514
4	3514·2	1·875	1·498	1·876	1·499
	T$_e$ = 43949·2 cm^{-1}		A$^1\Pi$ state		
0	44349·7	1·713	1·590	1·713	1·590
1	45141·5	1·767	1·552	1·767	1·552
2	45920·8	1·807	1·528	1·807	1·527
3	46687·5	1·841	1·509	1·842	1·510
4	47441·2	1·873	1·494	1·873	1·493
5	48181·5	1·903	1·480	1·903	1·480
6	48908·2	1·931	1·468	1·930	1·468
7	49620·7	1·958	1·457	1·958	1·457
	T$_e$ = 57688·0 cm^{-1}		C$^1\Sigma^+$ state		
0	58155·8	1·660	1·547	1·660	1·547
1	59083·8	1·709	1·511	1·709	1·511
2	60001·4	1·745	1·488	1·745	1·488
3	60908·6	1·775	1·470	1·775	1·470
4	61805·3	1·806	1·453	1·806	1·453
5	62692·4	1·828	1·442	1·828	1·442
	T$_e$ = 61229·5 cm^{-1}		D$^1\Delta$ state		
0	61678·5	1·672	1·555	1·672	1·555
1	62567·4	1·721	1·519	1·722	1·520
2	63444·0	1·758	1·496	1·758	1·497
	T$_e$ = 63689·4 cm^{-1}		E$^1\Pi$ state		
0	64149·6	1·665	1·550	1·665	1·551
1	65062·1	1·714	1·514	1·715	1·515
2	65964·0	1·750	1·491	1·750	1·490
	T$_e$ = 65795·6 cm^{-1}		F$^1\Pi$ state		
0	66271·9	1·656	1·543	1·656	1·543
1	67216·5	1·704	1·508	1·704	1·508
2	68150·3	1·739	1·485	1·739	1·485
	T$_e$ = 44813·2 cm^{-1}		$b\,^3\Sigma^+$ state		
0	45204·5	1·705	1·581	1·705	1·581
1	45975·5	1·760	1·543	1·760	1·544
2	46731·3	1·802	1·519	1·802	1·520
3	47471·6	1·839	1·501	1·840	1·500
4	48196·4	1·872	1·486	1·872	1·486
5	48905·7	1·904	1·473	1·904	1·471
6	49599·3	1·934	1·461	1·934	1·460
7	50277·4	1·961	1·450	1·960	1·450
	T$_e$ = 54957·7 cm^{-1}		$c\,^3\Sigma^+$ state		
0	55423·4	1·662	1·548	1·662	1·548
1	56347·4	1·711	1·512	1·711	1·512
2	57261·8	1·746	1·489	1·746	1·490
3	58166·6	1·777	1·471	1·778	1·470

method and authors' new method is excellent. It is therefore concluded that this new method can also be employed in the construction of potential energy curves in the absence of computational aids as it involves less complicated mathematical calculations.

The true potential energy curves (RKRV) for a molecular electronic states have been used to estimate the dissociation energies of diatomic molecules in a number of cases by fitting an empirical potential energy curve to the true potential energy curve (Steele *et al.*[18–19]; Singh, R. B. *et al.*[20–21], Sing, J. *et al.*[22]; Nair *et al.*[23]). The Hulburt-Hirsenfelder function has been shown to fit to a good extent the RKRV curves wherever these are known over a wide range of energy for a number of diatomic molecules (Steele *et al.*[9])

Hulburt-Hirschfelder function

$$V = D_e\left[(1 - e^{-ax})^2 + ca^3 x^3 e^{-2ax}(1 + abx)\right]$$

where

$$x = r - r_e$$

$$a = (K_e/2D_e)^{1/2}$$

$$b = 2 - 1/c\left[7/12 - \frac{1}{a^2 r_e^2}\left(\frac{5}{4} + \frac{5F}{2} + \frac{5F^2}{4} - G/12\right)\right]$$

$$c = 1 - \left(\frac{1}{ar_e}\right)(1 + F)$$

$$F = \frac{\alpha_e \omega_e}{6B_e^2} \text{ and } G = \frac{8\omega_e x_e}{B_e}$$

being five parameter function gives the best average results and in general gives the best or near the best fit of the potential for all the cases studied.

In the present work using Hulburt-Hirschfelder function the ground state $X^1\Sigma^+$ dissociation energy of AlF molecule is estimated as 56,587·5 cm^{-1} (7·016 eV which is in very good agreement with the value (6·85 ± 0·1 eV) suggested by Gaydon[24]. Using D_e, the ionization potential for AlF, is calculated assuming I of Al and D_e of AlF$^+$ from Gaydon[24].

$$\begin{aligned} I(\text{AlF}) &= I(\text{Al}) + D_e(\text{AlF}) - D_e(\text{AlF})^+ \\ &= 48{,}232{\cdot}9 + 56{,}587{\cdot}5 - 26{,}616{\cdot}8 \\ &\approx 78{,}203{\cdot}6\ \text{cm}^{-1} \end{aligned}$$

which is in good agreement with the value 80,000 cm^{-1} (9·918 eV) given by Barrow *et al.*[6].

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1. Rochester, G. D., *Phys. Rev.*, 1939, **56**, 305.
2. Rowlinson, H. C. and Barrow, R. F., *Proc. Phys. Soc., A*, 1953, 66, 437.
3. Naudé, S. M. and Hugo, T. J., *Cand. Jol. Phys.*, 1953, **31**, 1106.
4. Dosworth, P. G. and Barrow, R. F., *Proc. Phys. Soc., A*, 1955, **68**, 824.
5. Naudé, S. M. and Hugo, T. J., *Cand. Jol. Phys*., 1957, **35**, 64.
6. Barrow, R. F., Kopp, I. and Malmberg, C., *Physica Scripta*, 1974, **10**, 86.
7. Singh, J., Nair, K. P. R. and Rai, D. K., *Jol. Mol. Structure*, 1970, **5**, 492.
8. Fallon, R. J., Vanderslice, J. T. and Cloney, R. D., *Jol. Chem. Phys.*, 1962, **37**, 1097.
9. Steele, D., Lippincött, E. R. and Vanderslice, J. T., *Rev. Mod. Phys.*, 1962, **34**, 239.
10. Vanderslice, J. T., Mason, E. A., Maisch, W. G. and Lippincott, E. R., *Jol. Mol. Spectro.*, 1959, **3**, 17.
11. —, *Ibid.*, 1960, **5**, 83.
12. Lakshman, S. V. J. and Ramakrishna Rao, T. V., *Jol. Phys., B*, 1971, **4**, 269.
13. Herzberg, G., *Mol. Spectra and Mol. Structure—I*, Van Nostrand Company, New York. 1967,
14. Ramakrishna Rao, T. V. and Lakshman, S. V. J., *Jol. Quant. Spect. Radi. Trans.*, 1972, **12**, 1063.
15. Ramakrishna Rao, T. V. and Lakshman, S. V. J., *Ind. Jol. Pure and Appl. Phys.*, 1973, **11**, 539.
16. —, *Physica*, 1971, **56**, 322.
17. Chakraborty, B. and Pan, Y. K., *Applied Spectro. Rev.*, 1973, **7**, 283.
18. Steele, D., *Spectrochim. Acta*, 1963, **19**, 411.
19. — and Lippincott, E. R., *Jol. Chem. Phys.*, 1961, **35**, 2065
20. Singh, R. B. and Rai, D. K., *Cand. Jol. Phys.*, 1965, **43**, 1685.
21. —, *Ind. Jol. Pure and Appl. Phys.* 1966, **4**, 102.
22. Singh, J., Nair, K. P. R. and Rai, D. K., *Jol. Mol. Structure*, 1970, **6**, 328.
23. Nair, K. P. R., Singh, R. B. and Rai, D. K., *Jol. Chem. Phys.*, 1965, **43**, 3570.
24. Gaydon, A. G., *Dissociation Energies*, Chapman and Hall Ltd. (London), 1968.

STABILITY CONSTANTS OF SOME BIVALENT METAL CHELATES WITH 2, 4-DIHYDROXYVALEROPHENONE

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ABSTRACT

Proton-ligand stability constants of 2, 4-dihydroxyvaleroferore and the stability constants of its complexes with UO_2(II), Pd(II), Cu(II). VO(II), Ni(II), Co(II), Zn (II), Be (II), Mn(II) and Cd(II), have been determined employing Calvin-Bjerrum titration technique in aqueous 75% ethanol, 75% acetone and 75% dioxane (V/V) at 27° C. Thermodynamic formation constants have been obtained by extrapolation of the values at various ionic strengths (0·1, 0·05 and 0·01 M $NaClO_4$). The order of the stability constants has been found to be $UO_2 > Pd > Cu > VO > Zn > Be > Ni > Co > Mu > Cd$ which is in agreement with the Irving-Williams order of stability of bivalent metal complexes. The values of changes in free energy (△F), enthalpy (△H) and entropy (△S) have been calculated for the ligand. The value of $pK_m{}^H$ (minimum pK^H value at temperature θ° C) has also been calculated from Harned's equation.

CONSIDERABLE work has been reported on the determination of stability constants of metal complexes with *o*-hydroxybenzaldehydes[1–3] and *o*-hydroxyacetophenones[4–6]. However, similar studies on the metal complexes with 2, 4-dihydroxyvalerophenone have not been reported. This communication deals with the determination of stability constants of UO_2 (II) Pd (II), VO (II), Cu (II), Ni (II), Co (II), Zn (II), Be (II), Mn (II) and Cd (II) chelates with 2, 4-dihydroxyvalerophenone (DHV) in aqueous ethanol, acetone and dioxane medium at different ionic strengths using Calvin-Bjerrum technique as applied by Irving and Rossotti[7]. The value of free energy (△F), enthalpy (△H) and entropy (△S) have also been calculated for the ligand. The value of $pK_m{}^H$ value (at temperature θ° C) has also been calculated.

MATERIALS AND METHODS

pH measurements were carried out with Systronics pH meter (type 322). The system was calibrated at pH 4·05 with aq. potassium hydrogen phthalate buffer in the acid range, and at pH 9·20 with aq. borax buffer in the alkaline range. In aquo organic mixture the pH values were corrected by the use of Van Uitert and Hass equation[8]

$$-\log [H^+] = B + \log U_H$$

where B = pH meter reading and U_H = conversion factor. The conversion factor log U_H was obtained from the difference between the observed pH value 'B' and the theoretical value $-\log(H^+)$. An inert atmosphere was maintained by bubbling hydrogen gas through the solution. NMR of the ligand was recorded in $CDCl_3$ on Varian A.60 D instrument using TMS as internal reference.

2, 4-Dihydroxyvalerophenone[9] was synthesized by condensing *n*-valeric acid with resorcinol in the presence of anhydrous zinc chloride at 160° C. The product on recrystallization from chloroform gave colourless needles (m.p. 62° C; yield 60%). It was characterized by its NMR spectrum in $CDCl_3$. The triplet at 9·08 τ with J = 6·0 Hz was assigned to methyl group of the alkyl chain and the two methylene α, β to this methyl group appeared as multiplet 8·09 – 8·84 τ. The methylene protons, α to C= appear as triplet centered at 7·12 τ with J = 7·5 H The multiplet at 3·47–3·75 τ stands for two aromat protons *H*-3 and *H*-5. The *H*-6 proton appeared 2·39 τ as doublet with J = 9·0 Hz. Two D exchangeable hydroxy groups appeared as bro humps at 3·15 and – 2·86 τ.

All other chemicals were of B.D.H. AnalaR qualit Sodiun perchlorate was used to maintain the ioni strength constant. Standard carbonate-free sodiu hydroxide solution was prepared by the method Allen and Low[10]. Solution of perchloric acid (0·05 M was standardised by the sodium carbonate method Dioxane[11], ethanol and acetone were purified b standard methods.

For the evaluation of thermodynamic constant experiments were carried out in media of differen low ionic strengths, from these, an extrapolation t zero ionic strength was possible.

Calvin-Bjerrum Titration Technique

Following solutions (total volume 40·0 ml) were titrated against standard, carbonate free sodium hydroxide (0·05 M).

(A) 2·0 ml $HClO_4$ (0·05 M) + 4·0 ml $NaClO$ (1·0 M) + 4·0 ml H_2O + 30·0 ml solvent.

(B) 2·0 ml $HClO_4$ (0·05 M) + 4·0 ml $NaClO_4$ (1·0 M) + 4·0 ml H_2O + 10·0 ml ligand (0·01 M) + 20·0 ml solvent.

(C) 2·0 ml $HClO_4$ (0·05 M) + 4·0 ml $NaClO$ (1·0 M) + 1·5 ml H_2O + 2·5 ml metal solution (0·008 M) + 10·0 ml ligand (0·01 M + 20·0 ml solvent.

The ionic strengths 0·1 M, 0·05 M and 0·01 M were maintained by adding 1·0 M, 0·5 M and 0·1 M $NaClO_4$ respectively. The ligand solution was prepared in ethanol, acetone and dioxane which were used as solvents.

Proton-Ligand Stability Constants

The average number of protons associated with the ligand ($\bar{n}A$)) was determined from the acid ligand titration curve employihg the equation of Irving and Rossotti.

$$\bar{n}A = y - \frac{(v'' - v')(N^\circ + E^\circ)}{(V^\circ + v')\, T_{oL}^\circ}$$

where v' and v'' denote the volume of alkali required to bring the solution to the same pH in the titration of A and B respectively, T_{oL}° is the total concentration of the ligand, y the total number of dissociable protons attached to the ligand, N[illegible] the alkali, E° strength of acid (in 40·0 ml) and V° the initial total vo[illegible]

The calculation of protonation [illegible] carried out by plotting a graph bet[illegible] (the formation curve). The log pK[illegible] at half-integral value. The same ha[illegible] lated at different points using the [illegible]

$$\log K^H = pH + \log \frac{\bar{n}A}{1 - \bar{n}A}$$

The aveage values of log K^H are gi[illegible]

TABLE I

Protonation and formation constants of DHV chelates at 27 ± 0·2° *C*

Stability constants	75% Ethanol and 25% water (V/V)			75% Acetone and 25% water (V/V)			75% D[illegible] 25% w[illegible]	
	Ionic strength			Ionic strength			Ionic [illegible]	
	0·1 M	0·05 M	0·01 M	0·1 M	0·05 M	0·01 M	0·1 M	0·[illegible]
				H (I)				
log K_1^H	9·10	9·20	9·44	10·20	10·28	10·46	10·80	10[illegible]
				UO₂ (II)				
log K_1	7·20	7·35	7·73	8·60	8·71	8·90	9·50	[illegible]
log K_2	5·80	5·90	5·95	7·35	7·40	7·48	8·97	[illegible]
				Pd (II)				
log K_1	7·12	7·30	7·60	7·97	8·10	8·30	8·30	8[illegible]
log K_2	5·92	6·00	6·10	7·47	7·56	7·65	7·80	7[illegible]
				Cu (II)				
log K_1	6·37	6·50	6·68	7·80	7·95	8·25	8·25	8[illegible]
log K_2	5·57	5·70	5·85	7·05	7·11	7·20	7·40	7[illegible]
				VO (II)				
log K_1	6·70	6·81	7·00	7·65	7·80	8·04	8·00	8[illegible]
log K_2	5·30	5·33	5·40	6·65	6·70	6·84	7·20	7[illegible]
				Ni (II)				
log K_1	4·60	4·74	5·04	5·80	5·96	6·24	5·95	6[illegible]
log K_2	4·30	4·36	4·46	5·30	5·36	5·47	5·65	5[illegible]
				Co (II)				
log K_1	4·40	4·55	4·86	5·72	5·90	6·20	5·87	6[illegible]
log K_2	4·10	4·12	4·20	5·08	5·16	5·30	5·48	5[illegible]
				Zn (II)				
log K_1	5·07	5·20	5·40	7·25	7·30	7·45	7·43	7[illegible]
				Be (II)				
log K_1	4·70	4·83	5·00	5·83	6·00	6·25	6·07	6[illegible]
				Mn (II)				
log K_1	4·20	4·30	4·45	5·25	5·41	5·70	5·56	5[illegible]
				Cd (II)				
log K_1	3·95	4·03	4·20	4·65	4·80	5·05	4·81	4[illegible]

Metal-Ligand Stability Constants

The formation constants have been obtained by analysing the formation curves obtained by plotting $\bar{n}$ (the average number of ligands attached per metal ion) against pL (free ligand exponent). The values of $\bar{n}$ and pL were calculated from the equations

$$\bar{n} = \frac{(v''' - v'')(N^\circ + E^\circ)}{(V^\circ + v') \cdot \bar{n}_A \cdot T_{CM}^\circ}$$

and

$$pL = \log_{10}\left[\frac{\sum_{n=0}^{n=j} \beta_n^H \left\{\frac{1}{\text{antilog } B}\right\}^n}{(T_{CL}^\circ - \bar{n} \cdot T_{CM}^\circ)} \cdot \frac{(V^\circ + v''')}{V^\circ}\right]$$

where T_{CM}° is the total concentration of metal present in the solution, β_n^H is the overall proton ligand stability constant, B is the pH, v''' denotes the volume of alkali required to bring the solution to the same pH in the titration of (C) and other terms have usual significance.

RESULTS AND DISCUSSION

Proton-Ligand System

The maximum value of $\bar{n}_A$ was found to be less than 1·0, showing that only one step of dissociation is possible. Hence, DHV behaves as a monobasic acid. The values of log K^H at different ionic strengths in different media are given in Table I. The values of formation constants were determined by (*a*) interpolation to half $\bar{n}_A$ values and (*b*) the average value method, which are in agreement. It is probable that the acid dissociation corresponds to the phenolic OH ortho to the keto group in which the proton is replaced by metal ions during complex formation. An inspection of Table I shows that the pK^H value is least in aqueous alcohol and highest in aqueous dioxane system. This indicates that the acid dissociation of reagent increases in the order aq. ethanol > aq. acetone > aq. dioxane. A plot of pK^H values *vs* the reciprocal of dielectric constant showed a linear relationship.

Metal-Ligand System

The metal titration curves are well separated from the ligand titration curves indicating that the liberation of protons is due to chelate formation. The metal-ligand stability data (average values) at different ionic strengths in different media are reported in Table I. The values were calculated by various computational methods, *e.g.*, (*a*) half integral (*b*) least square method[12], (*c*) mid-point slope method[13].

The values of $\bar{n}$ in all the cases studied are always less than 2 which shows that there are only two complexes *viz.*, 1:1 and 1:2 in the system. In the complexes of Zn (II), Be (II), Mn (II) and Cd (II) the value of $\bar{n}$ is always less than one which is due to hydrolys In such cases only log K_1 has been calculated. I the case of VO (II), Ni (II) and Co (II), the values $\bar{n}$ were less than 1·5, hence log K_2 was calculat using the equation:—

$$2 \log K = \log K_1 + \log K_2$$

where

$$\log K = pL \text{ at } \bar{n} = 1 \cdot 0.$$

It has been found that log K_1 > log K_2 in all cases studied. The order of log K_1 of bivalent me complexes is found to be UO_2 > Pd > VO Cu > Zn > Be > Ni > Co > Mn > Cd. This or is in agreement with Irving-William[14,15] order of sta lity of bivalent metal complexes (Cu > Zn > Ni Co > Mn). The chelate of Cu (II) is found to relatively more stable than that of Ni (II) or Zn (I This might be due to the difference in the conf ration of Cu (II) chelate from those of Ni (III) Zn (II). It is probable that the copper forms sq planar chelates[16] using inner *dsp*^{2} orbitals, while ni and zinc form tetrahedral or preferably octahe chelates. The greater stability of zinc chelate may attributed to the electron delocalization of 3*d* orb of zinc through interaction with oxygen orbitals taking part in π-bonding and in the π-orbitals benzene ring. This is in agreement with the ob vations of Murakami *et al.*[17]. The order of stab of the chelates with respect of the solvent was dioxane > aq. acetone > aq. ethanol which is in reverse order of the dielectric constants of these vents (ethanol > acetone > dioxane).

THERMODYNAMIC PARAMETERS

The thermodyanmic formation constants (Table have been obtained by extrapolation of measured for tion constants to zero ionic strength from gra between log of stability constants against $\sqrt{\mu}$ (μ ionic strength).

The values of overall changes in the free ene ($\triangle$F), enthalpy ($\triangle$H) and entropy ($\triangle$S) for ligand calculated at three different temperatures an ionic strength 0·1 M $NaClO_4$ in 75% diox aq. medium using the following equations are giv in Table III.

$$\triangle F = -2 \cdot 303 \, RT \log K^{\mu=0}$$

$$\triangle H = 2 \cdot 303 \, R \frac{T_2 \times T_1}{T_2 - T_1} (\log K'' - \log K')$$

and

$$\log K = (\triangle S - \triangle H/T)/2 \cdot 303 \, R$$

where log K″ and log K′ are the log stabi constants at absolute temperatures T_2 and T_1 resp tively.

Harned *et al.* investigated the temperature variati of the acidity of acetic acid in water[18] and aq. dioxan and the acidity of other acids in water[20]. A parabo

TABLE II

Thermodynamic formation constants and free energies of metal chelates at 27 ± 0·2° C

Chelates	Ethanol (75%)		Acetone (75%)		Dioxane (75%)	
	log K°	$-\Delta F°$	log K°	$-\Delta F°$	log K°	$-\Delta F°$
Free-ligand	9·60	13·20	10·60	14·57	11·30	15·54
UO_2(II)	14·01	19·27	16·57	22·78	19·60	26·94
Pd (II)	13·98	19·22	16·20	22·28	17·17	23·60
Cu (II)	12·80	17·60	15·70	21·59	16·50	22·69
VO (II)	12·57	17·29	15·12	20·78	16·00	21·99
Ni (II)	9·82	13·50	12·00	16·52	12·62	17·35
Co (II)	9·34	12·85	11·85	16·29	12·13	16·68
Zn (II)	5·55	7·63	7·52	10·34	8·08	11·11
Be (II)	5·15	7·08	6·42	8·83	6·60	9·07
Mn (II)	4·55	6·25	5·88	8·08	6·25	8·60
Cd (II)	4·32	5·94	5·22	7·18	5·50	7·29

TABLE III

Protonation constants and the thermodynamic parameters at different temperatures and θ pK_m^H and ΔH calculated from Harned's equation for DHV at $\mu = 0 \cdot 1$ M

Temp. (° K)	pK^H average	$-\Delta F$ kcal/mole	$-\Delta H$ Kcal/mole average	ΔS cal./deg./ average	(θ° C)	pK_m^H	$-\Delta H^*$
293	10·92	14·66		24·7			7·51
300	10·80	14·85	7·42	24·7	212·00	9·09	7·63
308	10·65	15·03		24·7			7·69

ΔH^*, Using Harned's equation.

relationship between $\log K^H$ and t was found which could be expressed by the equation

$$pK^H - pK_m^H = C(t - \theta)^2$$

where $pK^H = -\log K^H$ at temperature t° C; pK_m^H—minimum pK^H value at temperature θ° C, and $C =$ constant ($5 \cdot 0 \times 10^{-5}$ deg^{-2}). Rearranging we get:

$$pK^H - Ct^2 = pK_m^H + C\theta^2 - 2C\theta t.$$

Thus a plot of $(pK^H - Ct^2)$ vs t should be a linear one having a slope of $-2C\theta$ and an intercept $pK_m^H + C\theta^2$ at $t = 0$. The values of $pK^H - Ct^2$ for DHV at various temperatures were plotted vs t and it was observed that the plot is linear; from which the value of θ and pK_m^H were obtained (Table III). The plot pK^H vs t is also linear. It has been observed by Harries and Wright[21] that this temperature range is so far removed from the plot of pK^H vs t and is the linear portion of the parabola.

ΔH values can also be obtained from the values of θ and t, using the equation: $\Delta H = 2 \cdot 303 \times 10^{-4} RT^2 (t - \theta)$ as suggested by Harned and co-workers[18-20].

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1. Calvin, M. and Wilson, K. W., *J. Am. Chem. Soc.*, 1945, **67**, 2003.
2. Jones, J. G., Poole, J. B., Tomkinson, J. C. and Williams, R. J. P., *J. Chem. Soc.*, 1958, p. 2001.
3. *Stability Constants*, Special Publication No. 17, Chemical Society, London, 1964.

4. Agreen, A., *Acta Chem. Scand.*, 1955, **9**, 39, 49.
5. —, *Swensk. kem. Tidskr.*, 1956, **68**, 185.
6. Perrin, D. D., *Nature*, London, 1958, **182**, 741.
7. Irving, H. and Rossotti, H. S., *J. Chem. Soc.*, 1953, pp. 3397.
8. Van Uitert, L. G. and Hass, C. G., *J. Am. Chem. Soc.*, 1953, **75**, 451.
9. Nogami, H., *J. Pharm. Soc.* Japan, 1941, **61**, 46.
10. Allen, N. and Low, G. W., *Ind. Engng. Chem. Analyt. Edn.*, 1933, **5**, 192.
11. Naidu, P. R. and Krishnan, U. R., *Trans. Faraday Soc.*, 1965, **61**, 1347.
12. Albert, A. and Serjeant, E. P., *Ionization Constants*, John Wiley and Sons, Inc., New York, 1962, p. 164.
13. Bjerrum, J., *Metal Ammine Formation in Aqueou Solution*, P. Hasse and Son, Copenhagan 1941.
14. Irving, H. and Williams, R. J. P., *J. Chem. Soc.* 1953, p. 3192.
15. — and —, *Nature*, London, 1948, **162**, 746.
16. Shah, J. R. and Patel, R. P., *Indian J. Chem.*, 1973, **11**, 606.
17. Murakami, Y. Nakamura, K. and Tolunaga, M., *Bull. Chem. Soc.*, Japan, 1963, **36**, 667.
18. Harned, H. S. and Ehlers, R. W., *J. Am. Chem. Soc.*, 1933, **55**, 652.
19. — and Kazanjian, *Ibid.*, 1936, **58**, 1912.
20. — and Embree, N. D., *Ibid.*, 1934, **56**, 1050.
21. Harries and Wright, G., *J. Inorg. Nucl. Chem.* 1969, **3**, 3149.

INDIRECT COMPLEXOMETRIC METHODS

Part III*. Determination of Thiocyanate and Ferrocyanide

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ABSTRACT

A convenient method for the estimation of thiocyanate ion is developed in which this ion is precipitated with known excess of copper sulphate after reduction to cuprous state and after reoxidation, the unreacted cupric ion is determined using EDTA. Similarly ferrocyanide is estimated by precipitation either as the cupric ferrocyanide or lead ferrocyanide and back-titrating the excess of the cation (cupric or lead) with EDTA. This method is found to have advantage over the earlier methods of estimation of these ions.

THE procedure of quantitative precipitation of an anion as its insoluble salt in the presence of an excess of a suitable cation and determination of the unconsumed cation by a complexometric method would appear to be a simple and obvious one. However a search through the literature showed that this principle has only been rarely exploited in practice. Attention in this direction seens to have been focussed largely in the case of only sulphate ion[1]. In the course of our investigations on the determination of anions by indirect EDTA methods, we had earlier shown that the sulphide ion[2] and the oxalate ion[3] may be conveniently determined in this way. These methods, while admittedly indirect and somewhat more tedious than the conventional procedures, have the advantage that they are applicable under conditions where the conventional methods fail. In the present communication, we describe two convenient EDTA methods for the determination of thiocyanate and ferrocyanide.

MATERIALS AND METHOD

Approximately 0·1 M EDTA solutions were prepared and standardised by established procedures[4]. These solutions were diluted suitably. Standard aqueous solutions of thiocyanate and ferrocyanide were prepared from BDH Analar Grade potassium thiocyanate and potassium ferrocyanide. Standard 0·01 M solutions of copper sulphate and 0·1 M lead nitrate were prepared using BDH Analar salts. These solutions were standardised independently by the usual procedures[4]. A buffer solution of pH 10 was prepared as specified[5]. As indicator for the complexometric titrations, murexide or Eriochrome Black T solutions, were prepared according to reported specifications[6]. Saturated sulphurous acid solution was freshly prepared by bubbling sulphur dioxide into distilled water. All other reagents used were of analytical grade.

PROCEDURE

(i) Determination of Thiocyanate:

Aliquots of copper sulphate solution (20 ml) were measured into a 400 ml beaker and diluted to 100 ml with distilled water. To these, 2 N hydrochloric acid (2 ml) and sulphurous acid (slight excess; 30 ml) were added. The mixture was heated to about 90° C and a measured aliquot (10 ml) of the thiocyanate solution was added dropwise, with stirring, from a burette. The precipitate formed was allowed to stand hot for 2 hours after which it was filtered and washed with dilute sulphurous acid solution. The filtrate and the washings were combined and boiled to expel the excess of sulphur dioxide. To this, conc. nitric acid (2 ml) was added and boiled to ensure that the entire cuprous ion was reoxidised back to the Cu (II) state. The solution was then cooled and the excess acid neutralised (pH7) carefully with sodium hydroxide solution. The buffer solution was then added dropwise until the precipitate formed redissolved completely to give a deep blue solution. Care was taken to avoid the addition of excess of the buffer[5]. Murexide indicator (2 drops) was added and the solution was titrated with standard EDTA solution until the colour changed from yellow (green in the presence of free cupric ions) to deep violet. Blanks were run concurrently. It was observed that no blank correction was necessary.

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* For Part II, *see* reference 3.

(ii) Determination of Ferrocyanide :

(*a*) *Using copper sulphate.*—The procedure was exactly similar to the one mentioned above for the determination of thiocyanate except that there was no need for the addition of sulphurous acid and the consequent reoxidation later.

(*b*) *Using lead nitrate.*—Measured aliquots of the standard lead nitrate solution (30 ml) were taken in a 400 ml beaker and diluted to 50 ml with distilled water. The solution was heated to about 90° C and a measured aliquot (5 ml) of the ferrocyanide solution was added dropwise, with stirring, from a burette. The precipitate formed was allowed to settle, then filtered and washed with water. To the combined filtrate and washings, tartaric acid (2 g) and the buffer solution (5 ml) were added. In case turbidity appeared, a little more tartaric acid has to be added. The solution was titrated with EDTA using Eriochrome Black T as the indicator until the colour changed from violet to clear blue. Blanks were also run concurrently and no blank correction was found necessary.

RESULTS AND DISCUSSION

The results of typical experiments are given in Table I.

As the experiments were carried out using Analar samples and yielded concordant values, no standard deviation was calculated.

It is seen from the results (Table I) that the present method has reasonably good precision and accuracy. The methods depend on the fact that the solubilities of cuprous thiocyanate, copper ferrocyanide and lead ferrocyanide are sufficiently low so that quantitative precipitation may be assumed. The solubility products of cuprous thiocyanate, copper ferrocyanide and lead ferrocyanide are $4 \cdot 8 \times 10^{-15}$, $1 \cdot 3 \times 10^{-16}$ and $3 \cdot 5 \times 10^{-15}$ respectively.

TABLE I

Anion determined	Amount of anion taken (in m moles per litre)	Amount of anion found (in m moles per litre)	No. of experiments done
Thiocyanate	100	100·2	3
Ferrocyanide (using copper sulphate)	10	9·96	4
Ferrocyanide (using lead nitrate)	100	100·0	4

It may be mentioned that, although good redox titrimetric methods are available for the determination of the anions mentioned above, these methods are highly susceptible to interferences either in the presence of other possible reductants or of interfering anions in the system. For example, although ferrocyanide can be estimated conveniently by permanganometric procedure, such a procedure will fail in the presence of other reductants. The presence of reductants which do not get precipitated by Pb^{2+} or Cu^{2+} will not possibly cause any interference in the present method. Similarly, the excellent argentimetric method available for the estimation of thiocyanate will be complicated if chloride or bromide is present and these halide ions do not cause any interference in the present method.

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1. Vogel, A. I., *Text-Book of Quantitative Inorganic Analysis*, Third Edn., ELBS and Longmans Green & Co., 1962, p. 425.
2. Ramamurti, G., Renganathan, K. and Ganesan, L. R., *Indian J. Chem. Education*, 1974, 4, 28.
3. —, — and—, *Curr. Sci.*, 1975, 44, 426.
4. Flaschka, H. A., *EDTA Titrations*, Second Edn., Pergamon Press, 1964, p. 67.
5. —, *Ibid.*, 1964, p. 80.
6. VOGEL, A. I., *Text-Book of Quantitative Inorganic Analysis*, Third Edn., ELBS and Longmans Green & Co., 1962, p. 446.

LETTERS TO THE EDITOR

HOMOGENEOUS AND HETEROGENEOUS REACTIONS ON THE DISPERSION OF A SOLUTE IN MHD COUETTE FLOW—II

RECENTLY Murthy and Murthy[1] discussed the influence of an applied magnetic field in the unsteady dispersion of a solute with simultaneous chemical reaction in a conducting liquid flowing in a channel under uniform pressure gradient. The authors[2] (hereafterwards referred as I) have considered the same problem for the flow of a viscous incompressible conducting liquid of uniform density ρ, confined between two horizontal non-conducting planes in the presence of a transverse magnetic field, when the upper and the lower planes $y = \pm d$ are moved horizontally with equal and opposite velocities $\pm U_0$ in the direction of the flow along the X-axis and there being no pressure gradient in the liquid. This case corresponds to the loading factor K (See for definition Sutton and Sherman[3]) equal to zero. The object of this note is to extend I to include the effects of K. The velocity profile in non-dimensional form is given by, in notation of I,

$$u(\eta) = \frac{\sinh M\eta}{\sinh M} + K\left(1 - \frac{\cosh M\eta}{\cosh M}\right), \tag{1}$$

where M is the Harmann number. We further obtain

$$u_x = \bar{u} - u = K\left(\frac{\cosh M\eta}{\cosh M} - \frac{\tanh M}{M}\right) - \frac{\sinh M\eta}{\sinh M}, \tag{2}$$

where $\bar{u}$ is the average velocity.

In case of diffusion with the combined homogeneous and heterogeneous reaction, we substitute u_x of eq. (2) in eq. (15) of Murthy and Murthy[1] and solving for C subject to the boundary conditions eq. (16) of their paper, we obtain

$$C = \frac{d^2}{DL}\frac{\partial C}{\partial \xi}\left[\frac{(M\cosh M + \beta\sinh M)\sinh \alpha\eta}{(\alpha\cosh\alpha + \beta\sinh\alpha)(M^2-\alpha^2)\sinh M} - \frac{\sinh M\eta}{(M^2-\alpha^2)\sinh M} - \frac{K\cosh\alpha\eta}{(\alpha\sinh\alpha + \beta\cosh\alpha)}\right.$$
$$\left.\times\left\{\frac{M\sinh M + \beta\cosh M}{(M^2-\alpha^2)\cosh M} + \frac{\beta\tanh M}{M\alpha^2}\right\} + K\left\{\frac{\cosh M\eta}{(M^2-\alpha^2)\cosh M} + \frac{\tanh M}{M\alpha^2}\right\}\right]. \tag{3}$$

Substituting for C from eq. (3) and u_x from eq. (2), we obtain Q of eq. (4) of I. Comparing this with Fick's law of diffusion, we find that the solute is dispersed relative to a plane moving with the mean speed of the flow with an effective dispersion coefficient D^* given by

$$D^* = \frac{d^5 K_1}{D^2} G(\alpha, \beta), \tag{4}$$

where

$$G(\alpha,\beta) = \frac{K^2}{2\alpha^2}\frac{1}{(\alpha\sinh\alpha + \beta\cosh\alpha)\cosh M}\left[\frac{M\sinh M + \beta\cosh M}{(M^2-\alpha^2)\cosh M} + \frac{\beta\tanh M}{M\alpha^2}\right]$$
$$\times\left[\frac{\sinh(M+\alpha)}{M+\alpha} + \frac{\sinh(M-\alpha)}{M-\alpha}\right] - \frac{K^2}{2\alpha^2}\left[\frac{2\tanh^2 M}{M^2\alpha^2} + \frac{2M + \sinh 2M}{2M(M^2-\alpha^2)\cosh^2 M}\right]$$
$$- \frac{K^2\tanh M\sinh\alpha}{\alpha^3 M(\alpha\sinh\alpha + \beta\cosh\alpha)}\left[\frac{\beta\tanh M}{M\alpha^2} + \frac{M\sinh M + \beta\cosh M}{(M^2-\alpha^2)\cosh M}\right] + \frac{K^2\tanh^2 M}{\alpha^4(M^2-\alpha^2)}$$
$$+ \frac{1}{4\alpha^2(M^2-\alpha^2)\sinh^2 M}\left[2 - \frac{\sinh 2M}{M} + \frac{2(M\cosh M + \beta\sinh M)}{\alpha\cosh\alpha + \beta\sinh\alpha}\right.$$
$$\left.\times\left\{\frac{\sinh(M+\alpha)}{M+\alpha} - \frac{\sinh(M-\alpha)}{M-\alpha}\right\}\right]. \tag{5}$$

When $\beta = 0$, corresponding to the case of diffusion with a homogeneous first order reaction only, we obtain,

$$F(\alpha) = \frac{K^2 M\sinh M}{2\alpha^3(M^2-\alpha^2)\sinh\alpha\cosh^2 M}\left[\frac{\sinh(M+\alpha)}{M+\alpha} + \frac{\sinh(M-\alpha)}{M-\alpha}\right]$$
$$- \frac{K^2}{2\alpha^2}\left[\frac{2M + \sinh 2M}{2M(M^2-\alpha^2)\cosh^2 M} + \frac{2\tanh^2 M}{M^2\alpha^2}\right] + \frac{1}{4\alpha^2(M^2-\alpha^2)\sinh^2 M}$$
$$\times\left[2 - \frac{\sinh 2M}{M} + \frac{2M\cosh M}{\alpha\cosh\alpha}\left\{\frac{\sinh(M+\alpha)}{M+\alpha} - \frac{\sinh(M-\alpha)}{M-\alpha}\right\}\right]. \tag{6}$$

When $K = 0$, we recover eqs. (6) and (7) of I from eqs. (5) and (6) respectively.

It is seen that the conclusions of *I*, *i.e.*, when $K = 0$ also hold good for other values of K, while as in Soundalgekar and Gipta's work[4], the dispersion coefficient $F(\alpha)$ or $G(\alpha, \beta)$ increases with increase in K for fixed values of all the other parameters.

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Waltair, *August* 10, 1976.

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1. Murthy, S. N. and Murthy, M. R. K., *Indian J. Phys.*, 1974, **48**, 634.
2. Ramana Rao, V. V. and Padma, D., *Curr. Sci.*, 1975, **44**, 803.
3. Sutton, G. W. and Sherman, A., *Engineering Magnetohydrodynamics*, McGraw-Hill, 1965, p. 351.
4. Soundalgekar, V. M. and Gupta, S. K., *Int. J. Heat Mass Transfer*, 1975, **18**, 527.

ANTIBODY FORMING CELL RESPONSE OF MICE TO DENGUE VIRUS GIVEN BY DIFFERENT ROUTES

Dengue type 2 virus produces a lethal infection in adult mice *via* the intracerebral route, but fails to produce morbidity or mortality when administered intraperitoneally to adult mice[1]. We have further noted that dengue virus remains ineffective when given intraperitoneally or intravenously to adult mice (unpublished data). Is this resistance to peripheral route of inoculation of dengue virus due to a greater production of interferon, or to a specific immune response? Interferon does not appear to be responsible for this resistance as it has been shown that the administration of 500 units of interferon had no effect on the outcome of dengue infection[2]. It was, therefore, considered worthwhile to investigate the immune response of mice following inoculation of the virus by different routes.

The study was carried out on an inbred strain of male albino mice, weighing 20 to 25 g (aged 4 to 6 months). The dengue type 2 virus (strain 23085) was used in the form of an infected mouse brain suspension. The virus was given in doses of about 10^3 LD_{50} intracerebrally (i.c.) to mice of group A, intraperitoneally (i.p.) to group B and intravenously (i.v.), through the tail vein, to group C. From each group, 5 to 7 mice were sacrificed daily between post-inoculation days 1 to 15 and the spleens collected aseptically. The splenic cells were squeezed out, washed thrice and suspended in MEM medium. The antibody plaque-forming cells were counted by the localized haemolysis-in-gel technique of Jerne *et al.*[3]. The coating of sheep RBC with the dengue virus was carried out using the technique of Russell *et al.*[4] Only direct plaque forming cells (PFC) were counted.

From each mouse, multiple slides were prepared, depending upon the count of the splenic cells. The mean values of PFC per 2×10^7 splenic cells (nucleated) of slides from 5 to 7 mice, studied daily are preseneted in Fig. 1. The mice given virus i.c. sickened and died by the 10th day, hence, in group A, the observation could be recorded upto day 9, only. In mice from group A, when given virus by the i.c. route, the PFC increased from $396/2 \times 10^7$ cells on day 1, reached a peak on day 7 ($716/2 \times 10^7$) and then decreased to $608/2 \times 10^7$ on day 9. The mice from group B, given virus i.p. showed 420 PFC/2×10^7 cells on day 1, which figure increased to 792 PFC on the 7th day. This number then declined until the 10th day, when the count was 440. The cells increased again and, on 15th day, the count was 584. Mice from group C, which were given virus by the i.v. route showed 384 PFC/ 2×10^7 splenic cells on day 1; this figure gradually increased to 748 PFC on 8th day. There was subsequently, a constant decline of PFC until the 15th day, when the count was 404.

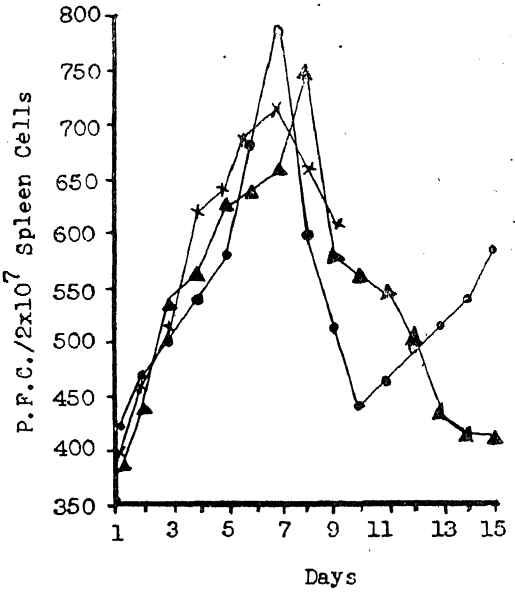

Fig. 1. Antibody plaque forming cells (PFC) in the spleen of mice given dengue 2 virus by i.c. (×—×), i.p. (●—●), or i.v. (▲—▲) routes.

The findings of the present study thus indicate that the immune response of mice administered dengue virus by different routes, as determined by the antibody plaque forming cells of the spleen, does not differ materially. Hence, a lack of the proper immune response does not appear to be responsible for the observed morbidity or mortality of mice when virus is given by the i.c. route. It appears that the replication of virus in the brain tissue is an essential factor leading to illness in mice.

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1. Thind, I. S. and Prince, W. H., *Amer. Jour. Epid.*, 1969, 90, 62.
2. Cole, G. A. and Wisseman, C. L., Jr., *Ibid.*, 1969, 89, 669.
3. Jerne, N. K., Nordin, A. A. and Henry, C., In *Cell Bound Antibodies*, Wistar Institute Press, Philadelphia, 1963.
4. Russell, S. M., McCahon, D. and Beare, A. S., *J. Gen. Virol.*, 1975, 27, 1.

TRANSFER OF JUVENOIDS AND INHIBITION OF EMBRYOGENESIS IN *DYSDERCUS CINGULATUS* (HETEROPTERA)

INHIBITION of embryogenesis by juvenile hormone analogues has been reported in the silk worm, *Hyalophora cecropia* (Riddiford, 1968), European corn bug, *Pyrrhocoris apterus* (Slama and Williams, 1966), and in locust, *Schistocera gregaria* (Novak, 1969). In the present investigation, the juvenile hormone analogue, P–169 (Ethyl-pivaloyl-L-alanyl-*p*-aminobenzoate) was tested on the red cotton bug, *Dysdercus cingulatus*, and its effects on oogenesis were studied.

The bugs were regularly reared (Judson *et al.*, 1976) and the freshly ecdysed adult females were topically treated with 1 μg/sp of the analogue. Its ID 50 value on the V instar nymphs of *D. cingulatus* has been shown to be 0·00004 μg/sp (Slama *et al.*, 1974). They were kept with freshly ecdysed untreated males and were mated. Controls were treated with 1 μl/sp of acetone only. On the third day, the females were dissected and their ovaries were examined and fixed for further histological studies. After removing the female on the third day another freshly ecdysed untreated female was introduced in its place. These insects mated normally and oviposited, but the eggs oviposited by this female failed to hatch.

The dissected ovaries of the treated females showed morphological differences when compared with the controls. Compound egg chambers (2 to 3 oocytes in each) were found in many ovarioles, in some ovarioles only one or two oocytes were developed instead of four to six as in the controls. Furthermore, a few ovarioles were in a degenerated condition (Divakar and Novak, 1974; Judson *et al.*, 1976). In the histological preparations also, the same effects found after treatment with 'Paper Factor' (Judson *et al.*, 1976), such as the compound egg chambers with continuous ooplasm were observed. This compound was thus found potent enough to cause sterility and contaminate the untreated ones through transfer of the analogue during copulation as reported by Masner *et al.* (1970) on *Pyrrhocoris apterus* with other synthetic juvenile hormone analogues. These results strongly support the statement of Williams (1967) that the juvenoids can be used as third generation pesticides and the auttors are strongly of the opinion that this compound could also be used in the field against plant bugs.

The authors are grateful to Dr. K. Slama, for providing the analogue and to the C.S.I.R., New Delhi, for the financial assistance to the first two authors.

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---

1. Divakar, B. J. and Novak, V. J. A., *Int. Congress on Regulation of Insect Reproduction*, Liblice, Czechoslovakia, 1974.
2. — and Kishen Rao, B., *Curr. Sci.*, 1975, 44, 555.
3. Judson, P., Divakar, B. J. and Kishen Rao, B., *Curr. Sci.*, 1976, 45, 867.
4. Novak, V. J. A., *J. Embryol. Exp. Morphol.*, 1969, 21, 1.
5. Riddiford, L. M. and Williams, C. M., *Proc. Natl. Acad. Sci.*, 1967, 57, 595.
6. —, *Proc. XIII Int. Congres of Entomology*, Moscow, 1968, p. 431.
7. Slama, K. and Williams, C. M., *Nature*, 1966, 210, 329.
8. —, Romanuk, M. and Sorm, F., *Springer-Verlag Wien*, New York, 1974, p. 193.
9. Williams, C. M., *Scientific American*, 1967, 217, 13.
10. Masner, P., Slama, K.; Zdarek; J. and Landa, V., *J. Econ. Entomol.*, 1970, 63, 706.

EFFECT OF GAMMA RAYS ON GROUNDNUT

INVESTIGATIONS have been reported in recent years on the use of radiation induced mutations in the improvement of groundnut[1,2]. Using X-rays Patil[3] isolated a large pod mutant, the tertiary branching mutant having increased pod setting. The present study was undertaken to investigate the effect of gamma rays on three varieties of groundnuts, namely, PVBM, Pollachi and TMV$_3$. One hundred dormant seeds of each of these varieties were irradiated with 20kR, 40kR, 60kR and 80kR of gamma rays. The treated seeds along with control were sown in the field in rows. Data collected are given in Table I from a study of M$_1$ generation on the three varieties.

Data on the percentage of germination were taken on the 10th day after sowing and the rest on the 35th day and on 95th day. In all the three varieties the decrease in percentage of germination of seed and pollen fertility were inversely proportional to the increase in the dose of gamma rays (Table I) indicating the lethality of the high doses on the life processes of the seedlings. These gradually became pale and died after 14 days. Data taken on 35th day indicate that treatment with 20kR did not reduce the height of the plant as well as the number of leaves per plant but at 40kR a considerable reduction in the height and in the number of leaves per plant was observed in the three varieties. Similar results were also obtained on the 95th day but in the variety PVBM, at 40kR treatment, the height of the plant and number of leaves per plant were equal to that of the plants at 20kR and the control. This may be due to the gradual recovery of the plants from the radiation effect. Pollachi and TMV$_3$ showed maximum reduction in height and slight decrease in the number of leaves per plant. The variations affecting the plant height have yielded short and dwarf varieties with condensed internodes.

During the period from the 35th to 95th day there was further mortality (Table I). Although

TABLE I

Sl. No.	Variety/Treatment	% of germinations	Seedling survival	After 35 days: Height cms ± S.E.	Average No. of leaves/plant	% of plants effected	After 95 days: No. of plants studied	Height cms ± S.E.	Average No. of leaves/plants	% of plants effected	No. of sterile plants	Pollen fertility %
1.	PVBM control	92	92	22·4±1·9	7·6	..	46	30·8±2·0	9·1	..	..	80
2.	20kR	84	84	20·5±1·9	7·7	44·0	42	30·4±2·6	8·2	64·3	..	71
3.	40kR	54	54	10·9±1·0	5·4	26·0	39	30·4±2·2	8·2	97·4	2	67
4.	60kR	47	nil	..	..	..	..	..	..	..	..	..
5.	80kR	37	nil	..	..	..	..	..	..	..	..	..
6.	Pollachi control	91	91	18·3±2·3	6·1	..	51	25·8±1·3	8·1	..	.	91
7.	20kR	89	89	18·4±1·9	6·2	19·1	44	27·7±1·1	8·0	27·3	..	75
8.	40kR	85	85	13·7±2·3	5·3	10·6	34	20·1±1·0	7·1	72·4	2	65
9.	60kR	49	nil		..	..	..	..	..	..	..	..
10.	80kR	33	nil	..	..	..	..	..	..	..	..	..
11.	TMV$_3$ control	88	88	18·8±2·7	7·5	.	44	27·0±2·9	8·2	..	..	97
12.	20kR	52	52	18·5±2·7	6·4	21·2	46	26·6±1·6	8·0	45·4	..	85
13.	40kR	40	40	14·0±2·4	6·0	20·0	24	20·1±1·6	6·2	83·3	2	65
14.	60kR	39	nil	..	..	..	..	..	..	..	..	..
15.	80kR	31	nil	..	..	..	..	..	..	..	..	..

the data collected on the 35th day indicated a higher percentage of plants with morphological and chlorophyll abnormalities at 20kR than at 40kR, the analysis on the 95th day revealed that the percentage of these plants was higher under 40kR treatment than at 20kR. This may be due to the recovery of plants at 20kR from the effects of radiations after the 35th day. Such a recovery was observed by earlier investigators also[3,4]. Apart from these observations leathery leaf, fused leaflet and imparipinnate leaf variants affecting the leaf character and Xantha, Albina, Dark green, Virescent and Striata were the ones found affecting the chlorophyll development. Sterile plants of two each in Pollachi and TMV_3 and two plants having sterile branches in PVBM were encountered at 40kR treatment.

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---

1. Gregory, W. C., *Oake Ridge Sym.*, 1957, 9, 36.
2. —, *NAS–NRC.*, 1961, 891, 461.
3. Patil, S. H. and Bora. K. C., *Indian J. Genet.*, 1963, 23, 47.
4. —, *Ibid.*, 1965, 26 A, 334.

SEROLOGICAL TESTS TO FIND OUT ANTIGENIC DIFFERENCES AMONG ISOLATES OF *XANTHOMONAS VESICATORIA* (DOIDGE) DOWSON AND SOME OTHER PHYTOPATHOGENIC BACTERIA

SEROLOGICAL techniques are used for identifying and typing pathogenic bacteria (Katznelson and Sutton, 1956; Lucas and Grogan, 1969; Otta and English, 1969; Charudattan *et al.*, 1973).

In the present investigation 28 isolates of *Xanthomonas vesicatoria* (22 from chillies, 3 each from tomato and *Dhatura*), 14 other *Xanthomonas* spp. and 5 non xanthomonads phytopathogenic bacteria were subjected to serological tests to find out antigenic differences among them. Antisera types I and II of *Xanthomonas vesicatoria* were obtained from Prof. R. E. Stall and R. Charudattan, University of Florida, USA. Agar gel double diffusion tests as described by Ouchterlony (1958) were used throughout these experiments. The medium consisted of 0·01 M PBS (Phosphate Buffered Saline, pH 7·2), Difco agar 1·5% to which 0·04 ml of 80% phenol per 100 ml of the medium was added as a perservative. Dilution of antisera wit saline water (0·85%) in the ratio of 1 : 2 was use throughout the experiments. After pouring th antigen and antisera in the respective wells, petr dishes were incubated at 8° C. Observations wer recorded at 12 hr. intervals upto 5 days for th formation of white precipitation bands between th antigen and aniserum wells.

On the basis of reaction with the type of ant serum/antisera and inability of the .isolates t react with either of the two antisera, the variou isolates were categorised into 4 groups. In thes reactions, two types of precipitation bands, *viz* arc like and fast moving near the antigens and ant sera wells respectively were formed similar to tho observed by Charudattan *et al.*[1].

Group I—reacted with type I antiserum, con prised of 18 chilli isolates, 2 tomato isolates, *dhatura* isolate. *Group II*—reacted with type antiserum comprised of 1 chilli isolate. *Group III*- reacted with type I and type II antisera compris of 2 chilli isolates. *Group IV*—did not react wi either type I or type II antisera, comprised of *dhatura* isolates and 1 chilli isolate. Reaction of isolates 2 each from chilli, tomato and *dhatura* wi type I antiserum is indicated in Fig. 1.

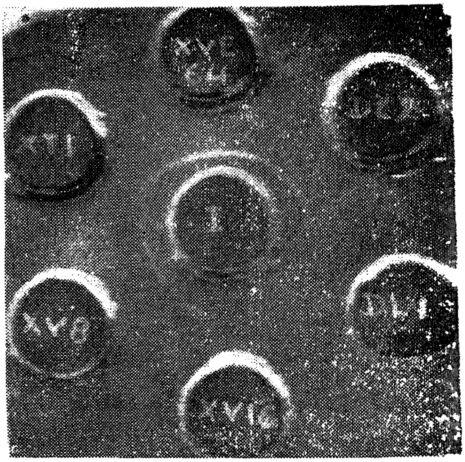

FIG. 1. Serological reactions of heated antig of *Xanthomonas vesicatoria* isolates; XB8 XV16 (from chilli), XT1 and XVE64 (from tom and DL1 and DL2 (from *dhatura*), with antise type I. Antigene of isolates DLI and XV16 not react while others formed a faast moving b near the antiserum well.

Out of 14 *Xanthomonas* spp. 5 species, *X. sesami*, *X. clerodendroni*, *X. argemoneae*, *X.* and *X. ricini* reacted serologically with type I serum and none with type II. Only *X. argemo* was found to react in the manner similar to that of *X. vesicatoria* in forming a fast moving band near the antiserum well with heated antigens and one arc like band near antigen well with non-heated antigens. Thus *X. argemoneae* may possibly belong to *X. vesicatoria* group and the 4 other species appear to share some common antigens with *X. vesicatoria* as they reacted with the antiserum prepared against *X. vesicatoria* though the fast moving band near the antiserum well by heated antigens was absent. Nine other species of *Xanthomonas, viz., X. translucens, X. oryzae, X. campestris, X. malvacearum, X. cymopsidis, X. azadirachtii, X. vignicola, X. amaranthicola, X. citri,* 2 species of *Pseudomonas, i.e., P. sesami, P. mangiferi-indicae, E. catorovora, Corynebacterium michiganense* and *Agrobacterium tumefaciens* did not react with either type I or type II antisera produced against *X. vesicatoria* isolates.

Thus, 9 species of *Xanthomonas,* 2 species of *Pseudomonas,* 1 species each of *Erwinia, Corynebacterium* and *Agrobacterium* are not serologically related to *X. vesicatoria* and serological specificity appears to be confined to generic level of phytopathogenic bacteria used in these experiments, and to some extent in species level.

Thanks are due to Prof. R. E. Stall and Dr. R. Charudattan, University of Florida, USA, for the supply of antisera and authorities of the Udaipur University, for facilities provided.

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1. Charudattan, R., Stall, R. E. and Batchelor, D. L., *Phytopathology,* 1973, 63, 1260.
2. Katznelson, H. and Sutton, M. D., *Can. J. Botany,* 1956, 34, 48.
3. Lucas, L. T. and Grogan, R. G., *Phytopathology,* 1969, 59, 1908.
4. Otta, J. D. and English, H., *Ibid.,* 1969, 59, 889.
5. Ouchterlony, O., In : S. K. Basel (ed.), *Progress in Allergy,* 1958, 5, 1.

STUDIES ON A MOSAIC DISEASE OF MUSK MELON (*CUCUMIS MELO* L.)

A SURVEY of virus diseases of musk melon (*Cucumis melo* L.) conducted in 1974 revealed two distinct types of symptoms, *viz.,* melon mild mosaic and melon ringspot mosaic. Detailed studies conducted on the mild mosaic disease (Fig. 1) are reported here.

The disease is characterised by mosaic mottling of the leaves in the form of irregular chlorotic and green areas on the leaves without any leaf distortion.

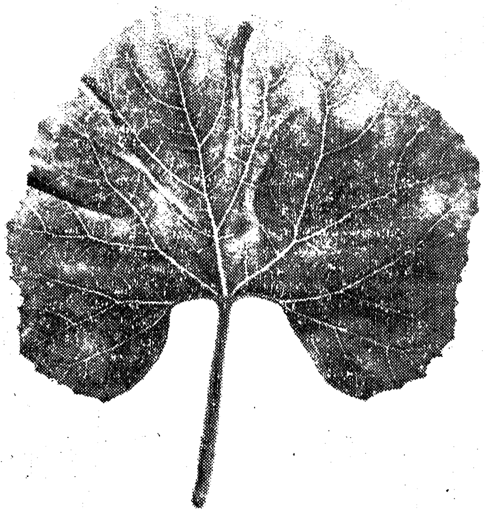

FIG. 1. Melon affected with mild mosaic.

The causal virus was found to be sap transmissible to cucurbitaceous hosts only. It infected bottle gourd (*Lagenaria sicerarea*), *Luffa cylindrica, Momordica charantia, Citrullus vulgaris, C. vulgaris* var. *fistulosus* and *Cucumis sativus* producing mosaic symptoms. The virus did not infect tobacco var. White burley or *Nicotiana glutinosa* but induced local lesions on *Chenopodium amaranticolor.*

The virus was found to be infective when heated to 90° C for ten minutes, at a dilution of 1 : 100,000 and also after storage for 60 days at room temperature.

The virus was purified using 8·5% butanol, M/20 phosphate buffer (pH 7·5) containing 0·1% thioglycollic acid and two cycles of differential centrifugation as reported by Shankar *et al.* (1971[1]. The electron micrographs (Fig. 2) revealed the virus particles to be rigid rods measuring 280 mμ × 15 mμ.

An antiserum of the virus was prepared by injecting white albino rabbits intramuscularly with purified virus preparations emulsified with Freund's adjuvant (Bacto) complete, thrice at weekly intervals followed by an intravenous injection with purified virus alone after a week and bleeding the rabbits 10 days after the last injection. The serum was separated from the red blood cells by decantation and low speed centrifugation at 7,000 rpm for 15 minutes and stored in 50% glycerol in a deep freeze.

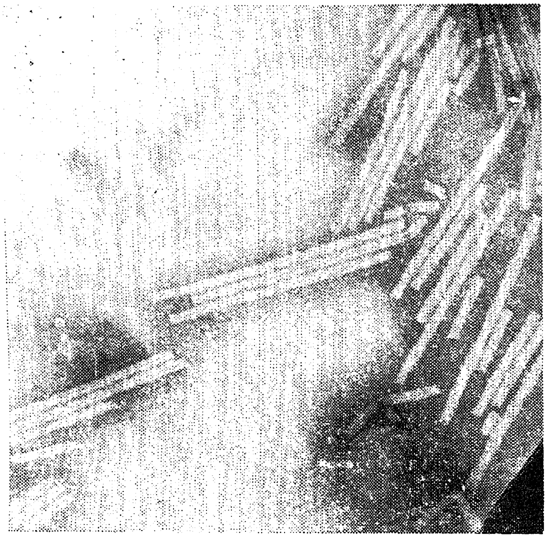

FIG. 2. Electron micrograph of melon mild mosaic virus stained wtih PTA, × 1,42,000.

The antiserum gave a positive precipitin reaction with diseased plant sap and purified virus preparations but not with healthy plant sap and had a titre of 1 : 2048. The antiserum also reacted with bottle gourd mosaic virus (*Cucumis virus* 2)[1], the culture of which has been maintained in the laboratory.

Thus the physical properties, host range, particle morphology and serological relationship suggest the causal virus to be cucumber green mottle mosaic virus[2] or *Cucumis virus* 2. Investigations on the melon ring spot mosaic virus are in progress.

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---

1. Shankar, G., Nariani, T. K. and Nam Prakash, *Indian J. Microbiol.*, 1971, 11, 43.
2. Smith, K. M., *A Text-Book of Plant Virus Diseases*, Longman Group Ltd., 1972.

GENETICS OF PIGMENTATION IN OKRA [*ABELMOSCHUS ESCULENTUS* (L.) MOENCH]

INHERITANCE of pigmentation in "Okra" [*Abelmoschus esculentus*, (L.) Moench] was studied in the cross Red Bhendi × Early Long Green, from observations of individual characters and their joint segregations in F_2. The nature of genes involved and their relationship including linkage have been worked out and the genes symbolized. The pigmentation on stem, petiole, and leaf vein is trigenically controlled, while petal veins and fruit pigmentation are controlled by a single pleiotropic factor.

Erickson and Couto (1963)[1] reported multiple factors for pigmentation on stem, petiole and single factor for fruit pigmentation. Kalia and Padda (1962)[3] suggested monogenic inheritance for fruit colour while Kolhe and D'Cruz (1966)[4] reported monogenic inheritance for stem, fruit and petal veins and trigenic for petiole pigmentation.

The cross 'Red Bhendi' × 'Early Long Green' was effected in *kharif* 74 and F_2 generation was studied in *kharif* 75. Observations on various plant parts were recorded at flowering and onwards. Tests for goodness of fit (Fisher, 1941)[2] and homogeneity test (Mather, 1957)[5] were applied and linkage values were worked out by product ratio method. The gene symbolization was adopted as suggested by Tanaka (1957)[6].

The segregation for pigmentation in the cross Red Bhendi × Early Long Green is presented in Table I.

The above data reveal that one of the complementary factors for stem is linked with recombination value of 7·12%. One gene is found common for petiole, petal veins and fruit pigmentation. One of the complementary factors is found common for petiole and vein while one of the two complementary factors is found linked with factor for petal veins with recombination value of 41·57%. The pleiotropic factor i_R is found common for leaf veins. No recombination is found for pigmentation in petal veins and fruit which is due to the same pleiotropic action.

Erickson and Couto (1963) reported multiple factors and Kolhe and D'Cruz (1966) monogenic inheritance for stem colour. The results obtained in these studies are different from those of earlier workers. Trigenic ratio of 45 : 19 and monogenic ratio, pleiotropy for petiole, petal veins and fruit pigmentation respectively have been confirmed in the present studies.

The factors S_{pa} & P_{vo} and P_{pa} & i_R have C.O. values 15·7 and 41·57% respectively.

TABLE I

F_2 segregation for pigmentation in a cross Red Bhendi × Early Long Green

Pigmentation on	Observed segregation		Ratio	X^2	Gene symbol		
	Pigmented	Non-pigmented					
Stem	245	48	54:10	0·1275	Sp_{al}	Sp_{al}	Sp_{a3}
Petiole	212	81	45:19	0·5846	P_{pa}	P_{pbl}	P_{pb2}
Leaf veins	254	39	55:9	0·1367	V_{pal}	V_{pa2}	
Petal veins and Fruit	220	73	3:1	0·0010	I_R		

Joint segregation for these five characters is given in Table II.

TABLE II

Joint segregations of five pigmentation characters in F_2 of Red Bhendi × Early Long Green

Characters	Observed segregation				Joint ratio	X^2
	XY	Xy	xY	xy		
Pigmentation on						
1. Stem (54:10)	Ob. 207·00	49·00	3·00	34·00	2430:450:1026:190	113·1852
with petiole	Ex. 173·82	32·18	73·39	13·61	Independent	
(45:19)	Ex. 200·00	47·21	6·00	39·79	Linkage, C.O. 7·12%	2·6553
2. Petal veins	Ob. 228·00	19·00	15·00	31·00	2970:550:486·90	115·9748
(55:9)	Ex. 212·34	39·43	34·76	6·47	Independent	
common gene	Ex. 233·38	18·06	18·06	23·50	813:63:63:81	3·2746
Petal veins	Ob. 216·00	30·00	00·00	47·00	48:6:0:10	0·3294
(3:1)	Ex. 219·75	27·47	00·00	45·78	Common gene	
II. Petiole (45:19)	Ob. 164·00	35·00	79·00	15·00	2475:1045:405:171	109·1587
with leaf	Ex. 176·98	74·81	28·97	12·24	Independent	
veins (55:9)	Ex. 175·15	30·90	76·65	10·30	612:108:268:36	3·4704
Petal veins	Ob. 162·00	47·00	43·00	41·00	135:57:45·19	23·9617
(3:1)	Ex. 154·51	65·25	51·51	21·73	Independent	
	Ex. 165·86	40·14	53·88	33·12	Linkage, C.O. 41·57%	5·3394
III. Leaf veins	Ob. 221·00	39·00	00·00	33·00	48:7:0:9	
(55:9) with	Ex. 219·75	32·05	00·00	41·20	Common gene	3·1461
petal veins (3:1)						

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---

1. Erickson, Homer. T. and Flavioap Couto, *Proc Amer. Soc. Hort. Sci.*, 1963, 83, 605.
2. Fisher, R. A., *Statistical Methods for Agricultural Workers*, Oliver and Boyd, London, 1941.
3. Kalia, H. R. and Padda, D. S., *Ind. J. of Genet.*. 1962, 22 (3), 248.
4. Holhe, A. K. and D'Cruz, R., *Ibid.*, 1966, 26 (1), 112.
5. Mather. K. *The Measurement of Linkages in* Heredity, Methuen and Co., London. 1957.
6. Tanaka, Y., *International Union of Biological Series B*, 1957, 30, 1.

INDIVIDUAL AND COMBINED EFFECT OF BACTERIAL AND ALGAL INOCULATION ON THE YIELD OF RICE

RICE field soil is a most efficient non-symbiotic nitrogen fixing ecosystem since its daily integrated nitrogen fixation is of the same order of magnitude as that of a symbiotic system. In submerged rice soils, biological nitrogen fixation is essentially an algal process[1] and artificial inoculation of the rice field soils with blue-green algae has been repeatedly found to benefit the rice crop both in terms of crop nutrition and productivity[2]. This preliminary communication deals with the effect of the commercial inocula of *Azotobacter chroococcum* and blue-green algae (obtained from Bafelab, Poona) on the growth and yield of two varieties of rice (Jaya and Tella Hamsa) under field conditions during June to November, 1975.

Table I shows the influence of the inocula on plant characters. *A. chroococcum* and blue-green algae alone significantly increased the number of effective tillers over no inoculation series. Although the panicle length and number of filled grains did not significantly differ between the *A. chroococcum* and blue-green algae treatments, these characters were strikingly influenced by the combined effect of *A. chroococcum* and blue-green algae. Individual application of *A. chroococcum* or blue-green algae resulted in the increase of 14–15% in the number of filled grains over the uninoculated series, while the combined inoculation of *A. chroococcum* and blue-green algae further increased it by 4–5% over the individual inoculation. Similar trend was observed with regard to the straw yield also.

TABLE I

Straw yield, effective tiller, panicle length, number of filled grains/panicle and test weight under different culture treatments

Cultures	Mean of two varieties under 4 nitrogen levels				
	Straw yield (q/ha)	Effective tillers (M^2)	Panicle length (cm)	No. of filled grains	Test weight (gms)
No culture	$48 \cdot 6_c$	$334 \cdot 5_d$	$19 \cdot 96_c$	$85 \cdot 75_c$	$22 \cdot 55_c$
A. chroococcum	$55 \cdot 6_b$	$345 \cdot 3_e$	$20 \cdot 65_b$	$97 \cdot 95_b$	$23 \cdot 30_b$
Blue-green algae	$58 \cdot 3_b$	$356 \cdot 5_b$	$20 \cdot 80_b$	$99 \cdot 17_b$	$23 \cdot 39_{ab}$
Azo + B.G.A.	$63 \cdot 5_a$	$371 \cdot 5_a$	$21 \cdot 20_a$	$104 \cdot 08_a$	$23 \cdot 68_a$
C.D.$_{0 \cdot 05}$	3·52	10·30	0·31	4·20	0·314

The experiment was laid out on a split plot design in plots of 3·75 × 2·8 m with 16 main treatments involving four levels of nitrogen (0, 50, 100, 150 kg N/ha) in the form of urea and four treatments involving (*i*) no culture, (ii) *A. choococcum,* (*iii*) blue-green algae and (*iv*) combination of *A. chrococcum* and blue green algae and sub treatments involving 2 varieties (Jaya and Tella Hamsa). Both the bacterial and blue-green inocula were suspended in water (600 gm in 6 litres of water) and the roots of 30 days old rice seedlings were dipped in them and transplanted in the experimental plots. The remaining portions of the inocula (@ 10 kg/ha) were sprinkled over the standing water in the respective plots, one week after transplantation.

In terms of grain yield, both the rice varieties significantly responded to increasing levels of nitrogen, although Jaya was more yielding than Tella Hamsa (Table II). While the application of *A. chroococcum* or blue-green algae significantly increased the grain yield over the uninoculated series, a combination of the two was better than the individual effect. The observed non-significant interaction between nitrogen and culture indicates the uniform beneficial effect of the cultures at every level of nitrogen. Besides nitrogen fixation the complementing effect of growth substance produced by these microorganisms may be responsible for the combined effect of Azotobacter and blue green algae. In the rice field ecosystem involving Azotobacter and blue-green algae, the requirements of the different components could be visu

TABLE II

Grain yield of rice (q/ha) under nitrogen levels, cultures and varieties

Cultures	Nitrogen levels kgs/ha								
	0		50		100		150		
	T.H.	JAYA	T.H.	JAYA	T.H.	JAYA	T.H.	JAYA	Mean
No culture	36·36	39·22	46·40	49·92	53·06	65·26	58·44	73·94	$52{\cdot}8_c$
A. chroococcum	42·66	46·34	52·62	60·46	58·04	70·36	60·44	76·58	$58{\cdot}4_b$
Blue-green algae	44·34	45·78	54·86	59·96	60·36	72·96	65·22	79·24	$60{\cdot}4_b$
Azo + B.G.A.	50·40	52·12	58·04	65·60	63·40	75·74	66·86	79·56	$63{\cdot}8_a$
Mean (V × N)	43·40	45·60	52·80	59·00	58·80	71·00	62·60	77·20	
Mean (N)	44·06		56·00		64·80		70·00		

Varieties· Tella Hamsa (T.H.)—54·46 Jaya—63·30.

Results of statistical analysis

Source of variation	*Significance* (5%)	$C.D._{0\cdot05}$
Nitrogen levels	Highly significant	3·2
Cultures	Significant	3·2
Nitrogen × cultures	Not-significant	..
Varieties	Significant	1·8
Varieties × Nitrogen	Significant	3·6
Varieties × cultures	Not significant	..
Varieties × Nitrogen × culture	Not significant	..

lised to take place through a recycling machanism as suggested by Venkataraman and Neelakantan[3].

The authors wish to record their sincere thanks to Dr. G. S. Venkataraman, Head, Division of Microbiology, IARI, New Delhi, for his thoughtful suggestions in writing this note.

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---

1. Stewart, W. D. P., (Ed.). *Nitrogen Fixation by Free-living Microorganisms*, Combridge University Press, London, 1975, p. 471.
2. Venkataraman, G. S., *Algal Biofertilizers and Rice Cultivation. Today and Tomorrow*, New Delhi, 1972. p. 71.
3. — and Neelakantan, S., *Phykos*, 1968, 7, 242.

STOMATA IN *OPHIOGLOSSUM PALMATUM* L.

THE epidermal structure of the Indian species of *Ophioglossum* was briefly described by Mahabale[1]. Pant and Khare[2] have studied the mode of development of stomata in four species of *Ophioglossum* and also in *Botrychium* and *Helminthostachys*. But as far as known, the epidermal features, which are being described here under, of *O. palmatum* are completely unknown. Statistical data on stomal index and other aspects are given for the first time.

The plants of *O. palmatum* (epiphytic gaint species) were obtained from U.S.A. (Fig. 1). Other species of *Ophioglossum* described in this paper were collected from several localities from Central India : *viz.*, Gwalior, Shivpuri, Narsinghgarh, Rewa, Ambikapur, Jagdalpur, Varanasi and Nainital. Excursion to these places have yielded eight species of the genus, *viz.*, *O. costatum* R. Br., *O. nudicaule* L., *O. petiolatum* Hook., *O. lusitanicum* L., *O. gramineum* Willd., *O. polyphyllum*. A. Braun apud Seubert, *O. vulgatum* L. and *O. costonudum* n. sp. Goswami and Khandelwal. The plants of *Botrychium* Swartz and *Helminthostachys* Kaulfuss were collected from Nainital and Gorakhpur respectively.

Epidermal features from mature leaves were studied from peels of the central portion. The peels were obtained by heating the material at 60° C for 15 to 30 minutes in the mixture of hydrogen peroxide, acetic acid water (4 : 4: 2 parts respectively). These were then stained with hematoxylin and mounted in glycerine.

TABLE I

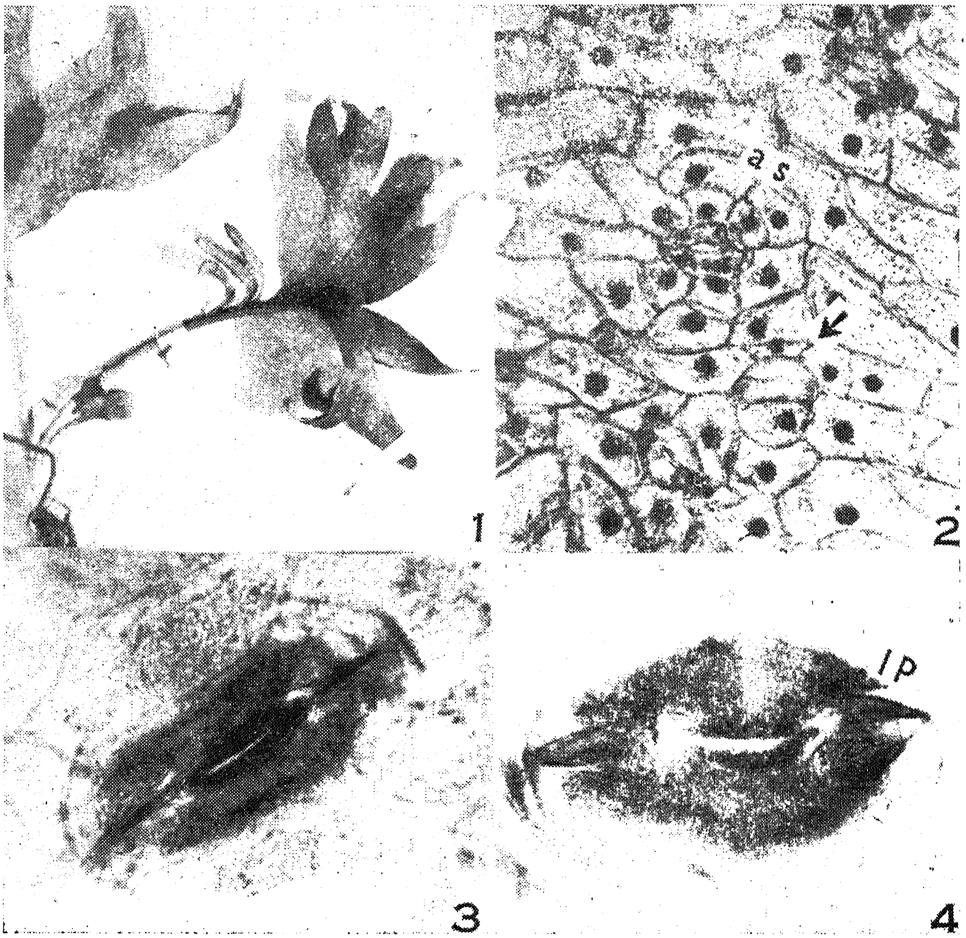
Epidermal features of the family Ophioglossaceae

Sl. No.	Name of species	Stomatal Index		Average size of epidermal cells in microns				Average size of stomata in microns			
				Upper		Lower		Upper		Lower	
		Upper	Lower	Length	Width	Length	Width	Length	Width	Length	Width
1.	*Ophioglossum costatum*	9·3	12·5	63± 9·9	33± 7·8	57± 19·18	31± 8·16	48±5·05	32±3·87	50± 3·74	38±6·48
2.	*O. nudicaule*	18·7	21·4	105±30·98	28± 7·80	94± 39·28	29±11·18	54±4·47	36±3·09	57± 4·84	36±5·05
3.	*O. gramineum*	10·8	12·6	108±22·80	45±19·02	115± 33·78	25± 8·77	57±4·12	33±3·13	67± 6·83	31±4·3
4.	*O. polyphyllum*	20·5	23·0	92±27·18	46±12·03	114± 26·94	31± 8·31	63±3·49	43±5·19	68± 7·60	41±5·39
5.	*O. petiolatum*	8·8	12·5	110±33·37	43±17·67	110± 39·52	26± 8·81	54±5·14	31±3·38	66± 8·31	34±4·09
6.	*O. lusitanicum*	13·6	18·1	129±35·60	30±22·67	156± 26·95	43±25·08	64±7·27	48±3·35	70± 3·69	40±5·51
7.	*O. vulgatum*	9·0	14·0	115±17·46	54±20·65	99± 55·26	52±30·20	75±6·39	55±3·49	76± 9·79	58±4·09
8.	*O. costonudum* (n.sp.)	9·6	12·5	66±23·89	34±12·16	84± 29·15	28± 6·45	47±7·34	36±5·8	56± 8·09	37±3·60
9.	*O. palmatum*	..	6·6	273±67·01	77±20·81	190±121·506	64±15·39	..	..	110±11·73	69±6·81
10.	*Helminthostachys zeylanica*	..	8·7	87±3[illegible]·97	29± 6·94	86± 36·73	18± 4·94	..	..	57± 7·17	31±3·49
11.	*Botrychium ternatum*	..	7·6	117±40·81	37± 9·39	97± 42·26	26± 7·21	..	..	78± 6·51	36±5·19

Mean of 10 values, ± = standard deviation.

Cells of epidermis are straight. They do not possess stomata on their upper surface. Both features are unlike other species of *Ophioglossum* (sinuous epidermal cells) but similar to *Helminthostachys* and *Botrychium*. The size of epidermal cells (273 × 77 μ) and the stomata (110 × 69 μ) are the largest among the Ophioglossaceae (Table I). But as expected, the number of stomata and epidermal cells on the lower surface are the lowest (Table I).

of perigene divided by radial walls before or after the formation of guard cells and increase their number. The meristemoid divides by two successive walls formed at right angles to each other cutting off two mesogene cells before the meristemoid functions as the guard cells mother cell. The guard cells of a mature stoma are surrounded by complete rings of subsidiary and encircling cells, each ring consisting of several cells (Fig. 2). The marginal cells of lower epidermis forming dome-like papillae such as noted in *H. zeylanica*[2] has not been observed in the present study.



FIGS. 1–4. Fig. 1. A plant of *O. palmatum* (× ¼). Fig. 2. Lower epidermal feature in *O. palmatum*. Note the anucleate guard cell mother cell (arrowed) and accessory subsidiary cells (*as*) (× 175). Fig. 3. Normal stomatal apparatus in *O. palmatum* (× 600). Fig. 4. Another stoma “showing lipped” projections (1*p*) in between the guard cells (× 600).

The mode of development of stomata in the present study essentially conforms to the pattern described by Pant and Khare[2]. Basically piper type, the stomatal development is perigenous in Ophioglossaceae. Frequently the neighbouring cells

In *O. palmatum* the stomata were seen to possess uniform guard cells (Fig. 3) or some of them had the “lipped” gaurd cells (Fgig. 4). These extensions from gaurd cells overlapped each other and gave a peculiar shape to the stomata. Such a state has not been reported in any member of Ophioglossaceae. It is very interesting to note that its stomata resemble more closely with that of *H. zeylanica* than with other species of *Ophioglossum*.

We are thankful to Mrs. Virginia Ault, California (U.S.A.), for sending the plants of *O. palmatum*.

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---

1. Mahabale, T. S., *Bull. Bot. Surv. India*, 1962, 4, 71.
2. Pant, D. D. and Khare, P. K., *Ann. Bot.*, 1969, 33, 132, 795.

SOME FUNGI HITHERTO UNREPORTED FROM INDIA

Two interesting fungi, *viz.*, *Guignardia musae* Racib. and *Synchytrium aureum* Schroet. were collected from Banana "Hari Chall" sweet variety and "Sowthistle" plants respectively, from Gorakhpur (U.P.). This forms the first report of their occurrence from India.

1. *Guignardia musae* Racib. (Conidial state Fig. 1A)

On the living leaves of *Musa sapientum* L. (Musaceae); Asuran, Gorakhpur; November 1975, leg. Y. N. Srivastava.

The fungus incites minute, rusty, dark brown, circular to oval blisters on the lamina (0·5–1·0 mm in diam.).

Sections of the infected leaves through the blisters show the Mycelium as endophytic, branched, septate and intracellular; Hyphae profusely ramifying in the intercellular spaces also (2–4 μ in thickness); Conidia roughly spherical (10–15 μ in diam.), thick-walled and produced in chains. The fungus with its perithecial state was recorded on the living leaves of *Musa paradisiaca* L., for the first time from Buitenzorg, Java[3].

2. *Synchytrium aureum* Schroet (Fig. 1B).

On the living leaves and stems of *Sonchus oleraceus* L. (Compositae); Kusmi forest, Gorakhpur; September 1975, leg. Y. N. Srivastava.

The fungus incites numerous, minute, granular galls unevenly distributed on the surface of the host stem and leaves (0·2–0·5 mm in diam.). Sections of the infected materials through the galls show host cells considerably enlarged in the infected region; mature prosori thick-walled. circular to oval (80–100 μ in diam.) but younger ones 7·0–7·5 μ in diam. and characteristically intracellular. Germinating prosori have also been observed; zoosporangia flatly oval (70–120 μ in di
measurements of prosori of the causal
differ from those reported by Cook[1] (
the same species recorded on *Lactu*
corresponds more to the original desc
Schroeter on *Lysimachia nummularia*.

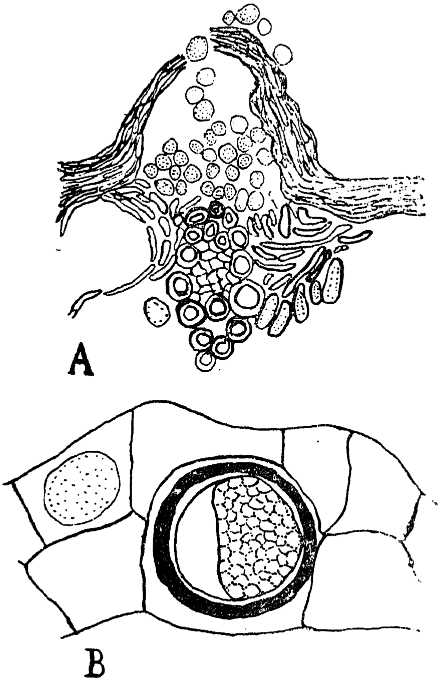

FIG. 1 A–B. Fig. 1 A. *Guignardia mu*
in host leaf section. Fig. 1 B. *Synchytriu*
young and mature prosori.

The specimens have been deposited in
I.M.I., Kew, at Nos. 199644 and 199642 re
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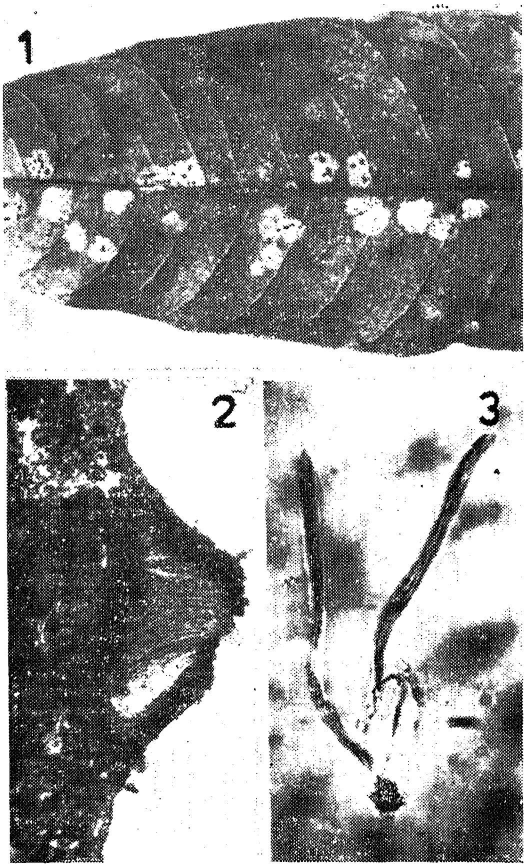
---

1. Cook, M. T., *Mycol.*, 1945, 37 (5),
2. Saccardo, P. A., *Syll. Fung.*, 1888, 7,
3. —, *Ibid.*, 1913, 22, 75.

OCCURRENCE OF *LOPHODERMIUM MANGIFERAE* ON MANGO LEAF IN VARANASI

A LEAF spot of mango (*Mangifera indica* L.) was collected in the old botanical garden of Banaras Hindu University in 1964 which, on examination, proved to be caused by *Lophodermium mangiferae* Koord. The fungus attacks fallen leaves producing amphigenous, whitish, scattered, irregular, 4·0–6·0 mm diam. spots (Fig. 1) with black speck-like ascocarps in the centre. A vertical section through the apothecium (Fig. 2) shows the paraphyses and asci within. The individual asci are cylindrical containing 8 filiform, multiguttulate ascospores (Fig. 3). The fungus was originally reported from Java (Koorders, 1907) and subsequently from Puerto Rico (Agri. Hand Book 165, 1960) and Brazil (Viegas, 1961). Apparently it is more widely distributed than the published reports indicate. One species of *Lophodermium*, *L. melaleucum* (Fr.) De Not., occurs on living leaves of *Rhododendron catawbiense* (Ellis and Everhart, 1892) but most others have been reported only from fallen leaves of flowering plants and conifer needles. This is the first report of the occurrence of *L. mangiferae* in India.



FIGS. 1–3. Fig. 1. Undersurface of mango leaf showing pale spots and dark apothecia of *Lophodermium mangiferae*, × 1. Fig. 2. Vertical section through an apothecium showing a narrow opening and a mass of asci and paraphyses, × 175. Fig. 3. Two asci with fasciculate ascospores within, × 650.

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---

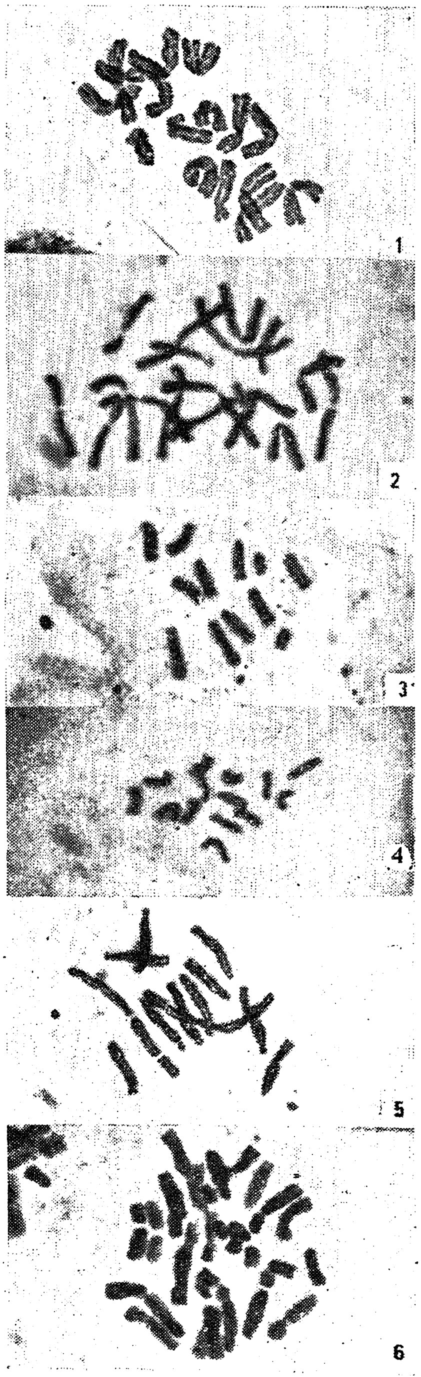
1. Ellis, J. B. and Everhart, B. M., *The North American Pyrenomycetes*, Published by the authors, Newfield, New Jersey, 1892.
2. U. S. Department of Agriculture Hand Book, 165, *Index of Plant Diseases in the United States*, Washington, D.C., 1960, p. 531.
3. Kooders, S. H., "Botanische Untersuchungen über einige in Java vorkommende Pilze, besonders über Blatter bewohnende, Parasitisch auftretende Arten," *Verh. Akad. Wet. Amst.* 1907, 13 (4), 1.
4. Viegas,, A. P., *Ìndice de Fungos da América do Sul.*, Secão de Fitopatologia, Instituto Agronômico—Campinas, 1961, p. 921.

ORTHO DICHLORO BENZENE: A NEW PRETREATING AGENT FOR CHROMOSOME ANALYSIS

THE application of chemicals for facilitating chromosome analysis inhibiting spindle and contracting chromosomes is well known[1,2]. While working with such pretreating agents, O-dichlorobenzene[3] (ODB) has been found to be more effective than the conventional agents in the root meristems of *Allium cepa, Allium sativum, Ornithogalum thyrsoides, Pisum sativum, Urginea indica* and *Vicia faba*. The following improved procedure has been evolved for obtaining well scattered chromosome configurations.

1. The root meristems are immersed for 30 minutes (*A. cepa, A. sativum* and *O. thyrsoides*) or for 60 minutes (*P. sativum, U. indica* and *V. faba*) in a partially (30–70%) saturated solution of ODB in water. The meristems are washed with water and fixed in Carnoy's fluid and stored in 70% alcohol or proceeded with directly.

2. The meristems are hydrolysed in NHCl for 8 minutes (*A. cepa, A. sativum* and *O. thyrsoides*) or 15 minutes (*P. sativum, U. indica* and *V. faba*) and stained in the modified feulgen reagent for 5 minutes[4] and aquashed in acetocarmine or 10% acetic acid.



FIGS. 1–6. Chromosome spreads of: 1. *Allium cepa,* 2. *Allium sativum,* 3. *Ornithogalum thyrsoides,* 4. *Pisum sativum,* 5. *Vicia faba* and 6. *Urginea indica.* (Figs. 1–6, × 1,200.)

ODB is comparatively cheap. As the saturat solution is cytotoxic, a further dilution by 30–70 is obligatory for desired results, unlike in the oth agents where saturated stock solutions are conveniently used. The effective duration of pretreatment is probably the shortest known for any suc chemical including its isomer *p*-dichlorobenzene Chromosome contraction and spreading is bett than in the other pretreating agents (Figs. 1–6) an compares well with colchicine, sometimes eve yielding diplochromosomal state.

The authors are thankful to C.S.I.R., India, an the authorities of the Berhampur University f financial assistance and facilities.

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---

1. Darlington, C. D. and La Cour, C. F., *Han ling of Chromosomes,* Allen and Unwi London, 1969.
2. Sharma, A. K. and Sharma, A. *Chromoson Technique—Theory and Practice,* Butte worths, London, 1972.
3. Lange, N. A., *Hand-Book of Chemistry,* M Graw-Hill Book Co., New York, 1956.
4. Sharma, C. B. S. R., Behera, B. N. an Velayudham, N., *Curr. Sci.,* 1976, **45**, 28.
5. Sharma, A. K. and Mookerjea, A., *Stain Tech* 1955, **30**, 1.

A NEW HOST RECORD FOR *PERICONIA PARASITICA* TILAK

SEEDLINGS of *Melia azadirachta* L. showing le spots were collected from St. Andrew's Colle Campus, Gorakhpur, during January 1976. Th fungus was identified as *Periconia parasitica* Tilak The symptoms and morphological characters of th pathogen are as follows:

Infection spots hypophyllous, light brow irregular (0·5–1·5 cm in diam.), with numero minute dark points. Mycelium is endophyt branched, septate and hyaline to brownish in colour Conidiophores straight as well as curved, 2- septate, fertile head separated by a septum, colo light brown, 150–170 μ long and 16–18 μ wid emerging in fascicles of 2–4; Conidia catenulat brownish, globose, verrucose and 12·5–17·5 in diam.

There are some variations in the number of sept in the conidiophores and measurements of th pathogen from the one originally collected by Tilak on *Desmodium laxiflorum* D.C. from Poona[2].

Pathogenicity of the causal organism has been tested.

Thanks are expressed to Dr. S. T. Tilak, Dr. K. S. Bhargava, Dr. Y. B. Singh and Dr. G. C. Srivastava for their help in various ways.

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---

1. Subramanian, C. V., *Hyphomycetes*, I.C.A.R., New Delhi, 1971, p. 322.
2. Tilak, S. T., *Mycopath. Mycol. Appl.*, 1958, 9, 194.

STUDY ON GENETIC VARIABILITY IN *CYMBOPOGON MARTINII* WATS. VAR. *MOTIA*

INDIA is the major producer of palmarosa oil, which is obtained by hydro-distillation of the grass—*Cymbopogon martinii* Wats. var. *motia*. In 1973–74 India exported 10·1 tonnes of palmarosa oil valued at Rs. 16·75 lakhs[1]. This crop grows wild in the drier regions of Maharashtra and in the neighbouring states[2]. Due to high geraniol content its oil is extensively used in perfumery and cosmetics. Very little work has been done on the genetic improvement of this species. The existing morphological variations in this species provided better scope for selecting a suitable genotype.

Forty-five different parental clones of *Cymbopogon martinii* var. *motia* were selected from the existing heterogeneous population grown at Aromatic and Mediciual Plants Garden of the Regional Research Laboratory, Bhubaneswar. The seeds were obtained from Lemongrass Research Station, Oddakali, Kerala, in 1969. The parental clones were grown under rainfed condition during 1975 kharif and the grass was harvested at full bloom stage. Ten effective tillers from each parent were studied. Observations on plant height, inflorescence length, fresh weight of stem, leaves, inflorescence and whole herb yield were taken (Table I). The number of tillers and fresh herb yield per bush were also recorded. The oil percentage of each parent were determined on fresh weight basis. The inflorescence density, the ratio of leaves *plus* inflorescence to stem and total amount of oil per bush were estimated. The ratio of weight of inflorescence (gm) to its length (cm) gave the inflorescence density. The oil percentage of leaves, stem and inflorescence were also determined separately (Table II).

The plant height ranged from 83·0 cm to 166·9 cm. It was observed that the dwarf plants were associated with thin stem. Since the stem contained very little oil (Table II) selection of a thin stemmed dwarf plant was aimed at.

TABLE I

Range of variation for different parental characters in palmarosa

Parental characters	Range of variation	Average of 450 observations
Plant height (cm)	83·0 –166·9	130·0
Inflorescene length (c)	13·2 – 56·8	31·2
Weight of inflorescene (g)	4·0 – 31·0	10·8
Weight of leaves (g)	5·0 – 34·9	17·3
Weight of leaves + inflorescence (g)	9·5 – 65·9	28·4
Weight of stem (g)	11·2 – 83·7	38·8
Ratio of leaves + inflorescence/stem	0·47 – 1·44	0·76
Inflorescence density (g/cm)	0·017 –0·080	0·035
Number of tillers/bush	40 –153	79
Percentage of oil on fresh weight basis	0·067 –0·67	0·41
Herb yield/bush (kg)	0·318 –0·825	0·536
Oil yield/bush (ml)	0·41 –4·38	2·11

TABLE II

Oil percentage of leaves inflorescence and stem of palmarosa

Plant parts	Range of oil percentage	Average oil percentage
Inflorescence	0·80 –1·00	0·85
Leaves	0·64 –0·86	0·76
Stem	trace	trace

The average weight and length of each inflorescence varied from 0·40 to 3·10 g and 13·2 cm to 56·8 cm respectively. RRL (B)–36 which had the highest inflorescence density of 0·068 g/cm gave the highest yield of 3·1 g per inflorescence. The number of tillers per bush ranged from 40 to 153. The yield of total herb increased with the increase in the number of tillers per bush.

The combined fresh weight of leaves and inflorescence varied from 9·5 g to 65·9 g, the highest being in RRL (B)–36. Since the oil content of leaves and inflorescence are higher than that of stem, plants having high leaves *plus* inflorescence to stem ratio were selected.

The herb yield per bush varied from 0·318 kg to 0·825 kg while the oil percentage and oil yield per bush ranged from 0·067% to 0·67% and 0·41 ml to 4·38 ml respectively. Progeny testing for correlating the herb and oil yield and oil percentage with the different morphological characters are in progress.

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---

1. *Monthly Statistics oj Foreign Trade in India*, 1974.
2. Guenther, E., *The Essential Oils*, 1950, **4**, 3–18

A NOTE ON A POLYHAPLOID DERIVATIVE IN *SORGHUM*

THE polyhaploid derivative of *Sorghum* IS 3122 showed a number of altered morphological characters when compared to the mother tetraploid plant. It was late to flower by about 10 days and the increase in plant height, leaf length and width were striking. The panicles produced were longer and there was an increase in length of primary branches and size of grains. In addition, increase in stomatal size and pollen diameter was also observed (Table I). The polyhaploid plant did not show any meiotic irregularity and regular bivalent formation was observed. Pollen fertility and seed set were comparable to the mother tetraploid plant.

TABLE I

Comparison of stomatal length and breadth and pollen diameter (in microns) in natural tetraploid IS 3122 ($2n = 40$) *and its polyhaploid derivative* ($2n = 20$)

Character	Polyhaploid ($2n$)	Tetraploid ($4n$)
Stomatal length	50·6±1·9	43·5±1·7
Stomatal breadth	28·5±1·7	25·0±1·4
Pollen diameter	47·7±1·4	36·5±1·3

± indicate standard deviation value.

A similar polyhaploid plant was also reported by Duara and Stebbins[2] (1952) in the F_6 generation of hybrid between the autotetraploid *S. vulgare* var. *sudanense* and *S. helpense* ($2n = 40$). However, this appears to be in contrast to the situation obtaining in autotetraploids induced through colchicine[1]. In general, induction of tetraploidy was reported to b associated with gigantism in some plant parts by man workers. However, the increase in various plan parts observed in polyhaploid derivative suggests tha the reduction in chromosome number might also lea to gigantism in plant parts. Basing on these observations it may be postulated that when the chromosom number is altered, either doubled or reduced to hal in a stabilized plant type it may lead to gigantism i plant parts.

The senior author is grateful to CSIR and UG for financial assistance.

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1. Bhaskara Rao, E. V. V., *Ph.D. Thesis*, Andhr University, Waltair, 1975.
2. Duara, B. N. and Stebbins, G. L. Jr., *Geneti* 1952, **37**, 369.

INTERFERTILITY STUDY OF *POLYPORUS GRAMMOCEPHALUS* BERK.

THE interfertility has created more interest aft Nobles[1] pointed out its significance in the taxono of fungi. So far, none has studied *Polyporus gramm cephalus* Berk. from this point of view. The prese communication gives the result of interfertility stu of *Polyporus grammocephalus*, a common woo rotting polypore of India.

The sporophore of *Polyporus grammocephalus* w collected from the campus of Burdwan Universi on a dead trunk of *Ficus benghalensis* L. (Bat.). Twen five monosporous cultures were made from the spo of this sporophore following the usual dilution meth on 2·5% malt agar slants. When each of the monosporous cultures showed good growth, th were checked carefully for clamp connections. Fin 20 monokaryotic cultures were taken into conside tion and were paired among themselves in all possi combinations on 2·5% malt agar slants. The cult tubes were left at room temperature (28 ± 2° C) about a fortnight and the line of contact between paired mycelia was examined from time to time.

The result of this examination is recorded Table I where (+) and (−) signs indicate presence or absence of clamp connections resp tively.

From Table I it will be evident that the myce fall into four groups which mean that the species the "tetrapolar" type of intefertility. The f sexual phases are designated as AB, ab, Ab and As expected in a tetrapolar species, clamp connecti

TABLE I

Pairings of 20 monosporous mycelia derived from a single sporophore of Polyporus grammocephalus *Berk.*

		AB				ab						Ab					aB				
		2	3	11	17	4	7	8	13	20	23	10	12	14	19	21	5	6	15	16	24
AB	2	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
	11	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
	17	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
ab	4	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	7	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	8	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	13	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	20	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	23	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ab	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
	14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
	19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
	21	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
aB	5	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–
	15	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–
	16	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–
	24	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–

occur only in pairings between AB × ab or Ab × aB, *i.e.*, between mycelia having no common allele.

The author is grateful to Dr. Anjali Roy for her guidance and to the University of Burdwan for providing Research Fellowship.

Botany Department,
Burdwan University,
Burdwan (W.B.), *April* 10, 1976.

A. B. DE.

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1. Nobles, M. K., *Can. J. Bot.*, 1958, 36, 883.

ANNOUNCEMENTS

AWARD OF RESEARCH DEGREES

Karnatak University, Dharwar, has awarded the Ph.D. degree in Mathematics to Shri Prakash Girimallappa Umarani; Ph.D. degree in Chemistry to Shri Shivanappa Khandappa, Haibatti; Ph.D. degree in Botany to Shri Ravindra Ramakrishna Hegde. Ph.D. degree in Chemistry to Sri Veluri Ananta Ramam.

The M.S. University of Baroda has awarded the Ph.D. degree to the following candidates for subjects mentioned against each: Shri Ramchandra Keshavlal Patel (Mathematics), Shri Kamalasanan M.N. (Physics), Shri Mammen Daniel (Botany), Shrimati Sunanda Prakash (Biochemistry); Shrimati Kailash Parimal Sanghvi (Chemistry), Srimathi Kumari Pratibha Devidayal Kathuria (Zoology).

Osmania University has awarded the Ph.D. degree to the following candidates for subjects mentioned against each: Shri Syed Gulam Samdani (Technology), Shri Nadimapalli Rama Krishna Raju (Chemistry), Kum. K. Lakshmi Prasunamba (Chemistry), Shri K. Subbash (Botany), Shri S. T. P. V. Jagannadha Swamy (Physics), Shri G. L. David Krupadanam (Chemistry), Shri C. V. Chalapati Rao (Chemistry).

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degree to the following fo. subjects mentioned against each: Shri V. Venkata Reddy (Zoology), Shri K. V. Siva Kumar (Physics).

Tamil Nadu Agricultural University, Coimbatore, has awarded the Ph.D. degree to the following: Shri T. R. Srinivasan, Shri K. K. Subbayya, Shri B. G. Rajasekhara, Shri S. Krishnamurthy, Shri V. Krishnasamy, Shri R. Bhaskaran, Shri P. Muthuvel, Shri A. Regupathy.

Utkal University, Bhubaneswar, has awarded the Ph.D. degree in Chemistry to Shri Trijama Ranjan Mohanty.

Berhampur University has awarded the Ph.D. degree in Chemistry to Shri Surendra Nath Mahapatra.

SHORT SCIENTIFIC NOTES

Effect of Hempa on the Last Instar Nymphs of *Chrysocoris purpureus* (Westw.)

The sterilizing effects of hempa (hexamethyl phosphoric triamide) on the reproductive system of various insects was studied by many workers[1–3]. But the details on the general behaviour and bionomics after treatment remains to be fully elucidated. The present communication contains a brief note on the toxic effects of hempa on *Chrysocoris purpureus*, W. (Pentatomidae, Hemiptera) and the side effects thereof.

The early fifth instar nymphs of both sexes were treated with hempa (0·0005, 0·001 and 0·01%) topically at the wing base and intraperitoneally at the pleural region near the 3rd abdominal spiracles (1 μl/nymph). After the treatment each batch of 20 nymphs were kept in a glass beaker covered by white muslin cloth, with a supply of fresh food (*Croton, sparsiflorus*, Euphorbiaceae) every day. Moulting and mortality counts were taken regularly at every 24 hours.

Mortality was very high when injected and 80% were dead in 5 days, while only 20% were dead in the topically treated lot. Survivors remained as 5th instars for quite a long period. In 10 days, only 10% of them in injection and 30% in topically treated were moulted into adults. While in the controls, 80% have emerged into adults in 10 days. Difficulty in moulting was also observed and mortality during the process of moulting was very common. Similar observations were made even at lower concentrations.

Further, the successfully moulted adults were observed for mating and oviposition by leaving them in converging crosses (treated males + virgin females; treated females + virgin males and treated males + treated females). Surprisingly no mating and oviposition were observed even after 10 days of their emerging into adults, while in the control mating and oviposition were observed with in 10 days.

Present studies indicate that the topical application of hempa is superior than the injection method. However, such treatment appears to interfere with the normal growth of both the sexes resulting in delayed moulting, reduced and malformed wings and notable changes in the behaviour of the adults.

The author is grateful to Prof. K. Venkata Ramiah, Director, Post-Graduate Centre, Warangal, for facilities and encouragement.

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Post-Graduate Centre,
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July 26, 1976.

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1. Chang, S. C. and Borkovek, A. B., *J. Econ. Ent.*, 1969, **62**, 1417.
2. Hafeez, M., Osman, M. F. and Erakey, M. A. S., *Ibid.*, 1970, **63**, 1161.
3. —, Abdul-Nasar, A. E. and Salama, H. S., *Ibid.*, 1970, **63**, 248.

Occurrence of *Achlya* Sp. on a New Host *Mystus* Spp., A Catfish

During the course of rearing experiments conducted on the commercial catfishes, *Mystus aor* (Ham.) and *Mystus seenghala* (Sykes), under the controlled laboratory conditions, occurrence of *Achlya* spp. was noticed.

Morphology of the Fungus

Mycelium diffuse, hyphae slender, branched. The zoosporangia were terminal and abundant, naviculate, 425μ–510μ in length and 30–85μ in breadth. The secondary zoosporangia were coming out from the basal end of the primary zoosporangia. The zoospores were formed within the sporangia in several rows and were encysted at the mouth immediately upon emergence. The encysted spores were 6·9μ–10·2μ in diameter. Oogonia terminal on the main hyphae, 41·1μ–45μ in diameter. No antheridia could be observed.

Authors are indebted to Dr. V. G. Jhingran, Director for his encouragement and to Shri A. V. Natrajan and Dr. A. G. Jhingran for their interest in the work.

Central Inland Fisheries B. C. JHA.
Research Sub-Station, R. N. SETH.
24, Pannalal Road, K. P. SRIVASTAVA.
Allahabad 211 002 (U.P.),
August 11, 1976.

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1. Jhonson, T. W., *The Genus* Achlya: *Morphology and Taxonomy*, Univ. Michigan Press., Ann. Arber, Mich., 1956.
2. Scott, W. W. and O'Bier, A. H., "Aquatic fungi associated with diseased fish eggs." *Progressive Fish Culturist*, 1962, 24, 3.

First Record of Swarm Occurrence and Incipient Breeding of *Locusta migratoria* L. in Kutch Distric

Jaswant Singh and Charan Singh, Jodhpur, notice a big size swarm of migratory locust (*Locusta migratoria* L.) over Baran (23°19′ N 68° 56′ E) of Kutc district on 1–10–1975 which is the first record fro desert area in India. Concentrated and incipie breeding by this swarm continued during Octobe November. Hopper emergence commenced durin

20th October and continued upto the end of November. Obviously the swarm split up into small parts for laying in ecologically suitable pockets. During October to December, unprecedented breeding occurred in Kutch district over a gross area of 500 sq. km. affecting 31 villages. The band formation was noted in most of the infested areas in advance instars and thick bands of size upto 80 square metres were seen with a typically gregarious behaviour during November and December. This is the first record of occurrence of gregarious pattern breeding in the desert area of India, as earlier, only scattered or concentrated breeding of this locust was recorded by Rao and Bhatia (1939) and Bhatia and Singh (1964). Control operations were initiated by dusting BHC 10%.

Locust Warning Organisation, JASWANT SINGH.
Locust Sub-Station, Jodhpur, CHARAN SINGH.
December 10, 1976.

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1. Rao, Y. R. and Bhatia, D. R., *Indian J. agric. Sci.*, 1939 9 (1), 79.
2. Bhatia, D. R. and Singh Charan, "Entomology in India". *Indian J. Ent.*, Silver Jubilee issue. 1964, p. 297.

A New Virulence of Rust Culture 104 of Leaf Rust of Wheat and Sources of Resistance

During 1974–75 crop season, an isolate of rust culture 104 of leaf rust (*Puccinia recondita* Rob. ex Desm.) was met with in a sample collected from Patna (Bihar) on HD 1 981 (Janak), a 2-gene dwarf wheat variety. Test isolate of the rust was observed to produce infection types similar to rust culture 104 on the International Differentials[1]. The two cultures, however, differed in their pathogenicity on "Thew", an auxillary differential being susceptible to test isolate and resistant to type culture 104 identified for the first time during 1971–72 crop season[2]. Because of the difference in their pathogenicity, the test isolate has been designated virulence 104–A. In the following crop season, *i.e.*, during 1975–76, the test virulence was met with in rust samples from the states of Rajasthan, Tamil Nadu and Uttar Pradesh, indicating its spread over a large area of the country.

Single spores were picked up from susceptible type of pustules developed on "Thew". Each single spore was inoculated on separate leaves of Agra local wheat. After the single spore cultures were established, each culture was further increased separately on susceptible check, *i.e.*, Agra local wheat. Each single spore culture was tested for patnogenicity on International Differentials along with auxiliary differentials. Each single spore culture produced identical infection types on each of the differentials and were similar to those produced by the test isolate, and the type culture 104, differing in pathogenicity only on "Thew", being susceptible to test isolate and resistant to type culture 104.

It may be added that intermediate infection types were produced on Carina and Webster by test virulence as well as the type culture 104. However, susceptibility is more predominant in the intermediate reactions during winter months (October–March) and resistance being predominant during summer months (April–September).

In order to have information on the resistant donors against the test virulence 104–A, a number of wheat cultivars were tested under glass house conditions in the seedling stage. The information on the resistant varieties of wheat, against test virulence, is given below:

Sonalika, Shailja, Kavkaz, Kiran, Torim-73, Cno-in-Bb, HD 2177, HD 2189, HD 2190, IWP 44, IWP 72, IWP 532, LSW 110, HW 142, UP 108, UP 241, UP 270, UP 278, UP 319, UP 368, CC 62, VL 428, E. 6160 E. 8678, Bajaura-1, WL 716, WL 950, HB 192, M 301–313, L 22, L 28, L 29, L 118, L 119, L 120, NRL-w-314, S 894, CPAN 1529, and CPAN 1555.

Wheat cultivars, *viz.*, IWP 500, IWP 503, HW 153, Burgas-2, Lr 9, Lr 19, NS 879/4, HD 2119, HD 2242, M301–3, M 301–7, E. 8667, CMM 67, KLM 4-8-2-1B, and KLM 4-8–1–3B, which were known to be resistant to all the virulences of leaf rust in India so far, were also found to maintain their resistance against test virulence 104–A also. These resistant stocks of wheat may be used in the hybridization programme or leaf rust resistance.

Authors wish to express their gratitude to Dr. A. B. Joshi, Director, Indian Agricultural Research Institute, New Delhi 110 012, for his keen interest during the course of these studies.

Indian Agricultural Research Institute, S. K. NAYAR.
Regional Station, SHEODHAN SINGH.
Flowerdale, Simla 171 002. S. K. SHARMA.
November 8, 1976. S. C. CHATTERJEE.
L. B. GOEL.

---

1. Johnston, C. O. and Mains, E. B., *U.S. Dept. Agric. Tech. Bull.*, 1932, **313**, 1.
2. Nayar, S. K., Goel, L. B., Singh, S., Sharma, S. K. and Chatterjee, S. C., *Indan J. agric. Sci.* 1974, **44** (8), 529.

Discovery of *Giraffokeryx punjabiensis* from the Dhokpathan Formation of Haritalyangar, Bilaspur District (H.P.)

The earliest fossil giraffids known to the Palaeontologists come from the miocene horizons of Africa. In India, fossil giraffids belonging to the genera *Sivatherium Giraffa*, *Giraffokeryx*, *Vishnutherium*, *Bramatherium* and *Hydaspatherium*, etc., are known to occur in the Śivalik sediments which constitute the foot-hill zone of the Himalaya. India was rather a centre of adaptive radiation of the giraffes during the sedimentation of Śivaliks.

The present communication places on record, for the first time, the occurrence of *Giraffokeryx punjabiensis* from the Dhokpathan Formation exposed in the Haritalyangar basin (H.P.). The same formation in this tract is conformably overlain and underlain by Tatrot and Nagri Formations respectively—the later being highly fossiliferous and particularly known for yielding rich primate fauna[1–2].

The Dhokpathan Formation, which has yielded the species under discussion, lithologically consists of greyish and pinkish clay alternating with brownish and greyish medium to coarse grained sand-stone and conglomerate beds in this terrain. Dhokpathan beds earned world-wide popularity when they yielded a well-known primate *Gigantopithecus bilaspurensis*[2–4] in addition to certain mammalian forms including species of *Crocuta*, *Hipparion*, *Sus*, *Rhinoceros*, *Dinotherium*, *Trilophodon*, *Pentalophodon*, *Stegolophodon*[1], fish remains[5] and also an insectivore[6].

Girraffokeryx punjabiensis was earlier known to occur only in the Nagri Formation of the Haritalyangar basin. The present finding extends the upper limit of this species upto Dhokpathan in this area. In the northwestern foot-hill zone of the Himalaya, the genus is also known from the Middle Śivalik units exposed horthwest of Jawalamukh, Lower Śivliks of Ramnagar, and Lower and Middle Śivaliks of Pakistan.

The present discovery consists of an isolated but a typical maxillary second molar which is nearly squarish (length 32·00 mm; width 27·00 mm) depicts a Selenolophodont pattern on a bunodont crown with rugose enamel. The structure and wear of the present molar confirms *Giraffokeryx punjabiensis* to be a forest browser like modern okapi.

Along with other associated mammalian finds, the present discovery further confirms the presence of forests interspersed with open woodlands and grassy plains with meandering streams and river banks during the sedimentation of Dhokpathans, in this basin.

Department of Anthropology, S. R. K. CHOPRA.
Panjab University, R. GAUR.
Chandigarh 160 014, I. J. SUNEJA.
August 12, 1976.

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1. Prasad, K. N., *Pal. Ind. Geol. Surv. Ind.*, 1970, **39**, 1.
2. Chopra, S. R. K., *Proc. 66th Ind. Sci. Congr.* 1974, 1.
3. —, *Akashwani*, 33, 9.
4. Simons, E. L. and Chopra, S. R. K., *Posilla*, 1969, **138**.
5. Chopra, S. R. K., Suneja, I. J. and Kaul, S., *2nd Conf. Ind. Assoc. Phy. Anthrop.*, 1975 (Abs.).
6. Sahni, A. and Khare, S. K., *Jour. Geol. Soc. Ind.*, 1976, **17**, 144.

Chromatographic Examination of Anthocyanins in *Lathyrus sativus* Linn.

In the present note, the anthocyanins in Kesari pulse were studied by paper chromatography and spectrophotometric examination by a Bausch and Lamb spectronic 20 type spectrophotometer. Fresh and sound quality of Kesari pulses were powdered to pass through 25 mesh sieve. About 0·1 gm of the powder was placed in 25 ml cold methanol containing 1% hydrochloric acid for 12 hours. The filtrate containing the pigment was concentrated *in vacuo* below 50° C and stored at 0° C. A few microlitres of this was spotted on Whatman No. 3 filter paper and chromatographed in ascending type for 18 hours, unidimensionally, using *n*-butanol : acetic acid : water = 4:1:5 by volume, upper phase and *n*-butanol : 2N HCl = 1:1 by volume, upper phase as developing solvents.

The chromatogram showed two spots which were identified on the basis of their R_f values, colour reactions and comparison with authentic samples as cyanin (cyanidin, 3:5 diglucoside) ($R_f \times 100$) values are 27 (BAW) and 10 (Bu-HCl) respectively and Pelargonin (Pelargonidin 3:5 diglucoside) ($R_f \times 100$) values are 33 (BAW) and 15 (Bu–HCl) respectively. Authentic samples of cyanin and pelargonin were prepared from red roses[1] and pelargonium flowers respectively and cochromatographed with the pigment. The ($R_f \times 100$) values of the pigment matched with those earlier reported by Harborne[2]. The presence of cyanin and pelargonin in the pigment under study were further confirmed by their acid hydrolysis with NHCl. The aglycones with sugar residues were formed from the glycosides on hydrolysis which were also chromatographed and later subjected to spectrophotometric examination. The aglycones so formed were identified as cyanidin ($R_f \times 100$) = 69 in BAW and absorption maxima 536 mμ in MeOH–HCl and pelargonidin ($R_f \times 100$) = 78 in BAW and absorption maxima 522 mμ in MeOH–HCl. However, the sugar residues were not studied.

The authors are grateful to the authorities of the Corporation of Calcutta for providing facilities for the work.

Food Section, J. K. SARKAR.
Central Laboratory, P. K. BANERJEE.
The Corporation of Calcutta,
July 7, 1976.

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1. Seshadri, T. R., In *The Chemsitry of Flavonoid Compounds*, Ed. Geissman, T. A., Pergamon Press, London, 1962, p. 10.
2. Harborne, J. B., *Phytochemical Methods*, Chapman and Hall, London, 1973, p. 63.

REVIEWS AND NOTICES OF BOOKS

Energy (Vol. III): Nuclear Energy and Energy Policies. Edited by S. S. Penner. (Addison–Wesley, Reading, Massachusetts 01867), 1976. Pp. xxxii + 713. Price: Hard Binding $ 28.50; Paper Binding $ 16.50.

The volume under review is the third of a series of lecture notes on Energy and deals with nuclear energy technologies and with energy policy. It differs from the preceding volumes in that the contents include contributions by separate authors. The subject-matter under discussion is, however, so diverse that multiple authorship appears to be necessary in order to achieve authoritative coverage.

The book opens with a chapter (Chapter 21) on Nuclear Fission Energy. A good portion of the chapter is descriptive followed by an Appendix wherein the theoretical basis for Nuclear Engineering Design Analysis is discussed. Topics covered include Pressurized Water Reactors (PWR), Boiling Water Reactors (BWR), High Temperature Gas (Helium)-Cooled Reactors (HiGR), Heat-power cycles, Process Heat, Design Requirements, and Nuclear Safety.

The Second Chapter of the book (Chapter 22) deals with Breeder Reactors, including the Liquid Metal Fast Breeder Reactor (LMFBR) and Helium-gas-cooled Fast Breeder Reactor (GCFR). Next chapter of the book (Chapter 23) is devoted to controlled Thermonuclear fusion. The topics discussed are physical processes in Nuclear fusion, magnetic confinement and stability of magnetically confined plasmas, transport processes in confinement plasmas, current status of magnetic confinement. Analytic estimates for energy production, compression by shocks, Quasi-adiabatic compression of sphere and shells, estimation of Neutron production shell implosions and Lasers for fusion.

Environmental Aspects of Nuclear Power Applications are discussed in Chapter 24 of the book. Radiation protection standards accidents involving nuclear fusion reactors, Fuel reprocessing, Nuclear waste disposal, estimates of environmental and safety aspects of fusion are some of the topics dealt with.

The next two chapters (Chapters 25 and 26) are devoted to Nuclear strategies and Energy policies and include such topics as Programming of Nuclear-Reactor Development to produce Desired Electricity-generating capacity, Population and Energy needs, Monitoring the Global Impact of Energy use, etc.

There are nine contributors, six from academic field nad three from industry and the book is a product of their lecture notes delivered at the Energy Centre, University of California, San Diego, to the UCSD graduate students. I have no hesitation in recommending the book, both as a text-book and a reference volume on "Energy". It should find a place in every technical library.

H. N. RAMACHANDRA RAO.

Journal of Power Sources, Vol. 1, No. 1, Editor D. H. Collins. (Elsevier Sequoia, S. A., P.O. Box 851, CH-1001 Lausanne, Switzerland), 1976. Pp. 108. Price: Annual Subscription: SFrs. 140.00; $ 54.00.

The Journal of Power Sources is intended to bring together work from the many scientific disciplines, technologies and branches of engineering which relates to primary and secondary batteries, fuel cells; solar power sources, thermionic, thermoelectric and isotope generators and other devices for the non-mechanical generation, storage and conversion of electric power.

The Journal aims to publish original contributions describing recent scientific advances, new techniques, materials and production methods and processes relevant to the various power sources. Emphasis is also placed on the efficient use and unusual applications of eixsting types of power sources.

The field of power sources differs from most other science and technologies in being extremely interdisciplinary. The Journal will be of special interest to the workers in the field as it brings together within one cover as many aspects of the field as possible, so as to give a full picture of what is being developed, how it is being used, why some systems are not available yet and why others have failed in practice. In this first issue, papers dealing with materials for lead acid batteries, for fuel cells and solid-state batteries are presented; also included are papers on development aspects of fuel cells, on manufacturing alkaline cells, a zinc-air battery for vehicle propulsion, solid electrolyte batteries and on a means of improving the manganese dioxide electrode and estimating its capabilities.

The Journal has an International Editorial Board and is published from Switzerland. Its frequency is Quarterly (4 issues per volume) and the subscription rate is SFr 140.00 per volume.

The Journal is recommended for all technical libraries.

H. N. RAMACHANDRA RAO

Horizons in Biochemistry and Biophysics (Vol.2). Edited by E. Quagliariello, F. Palmieri and T. P. Singer. (Addison-Wesley Publishing Company), 1976. 340 pages.

The 'life sciences explosion' during the past two decades has led to such a continuing deluge in the literature that the experimental research scientist is hard put to keeping abreast of developments in his own field of specialization. This leaves him little time to familiarize himself wtth developments elsewhere. Publications like the book under review have a noteworthy function to perform in this regard. Short essays on topics of active pursuit written by persons, who have contributed to the development of the topic, would go a great way in satisfying the curiosity of both the "aspiring" and the "established" scietists. Viewed in this light, the present volume is quite topical in that six of the total of nine essays deal with transport which is at present a 'hot' field in membrane research. Though terms like "insaturation" are met with occasionally, the serveys are immensely readable. But the treatment on the whole is too elementary to satisfy the discerning scientist.

The essay on metabolite transport into mitochondria is up to date and well written. However, no effort appears to have been made to evaluate transport activity in terms of energy expenditure and to discuss the thermodynamic aspects of ion transport across membranes. This criticism should apply to almost all the articles in this volume. These deficiencies not withstanding, this volume will be a useful addition to all scientific libraries and will make profitable reading for the 'busy' scientist.

C. K. R. KURUP.

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SPECTROPHOTOMETRIC STUDIES ON PLATINUM(IV)-PHENOTHIAZINE COMPLEX

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ABSTRACT

Phenothiazine forms an yellowish-brown complex with platinum (IV) at a pH value of 0·5–3·5. The colour complex exhibits absorption maximum at 440 nm. A twenty-four-fold molar excess of the reagent is necessary for the full development of colour intensity. Beer's law is valid over the concentration range 0·2–3·4 ppm. The sensitivity of the reaction is 0·02 μg/cm^2 and the molar absorptivity is $9\cdot72 \times 10^3$ l mole^{-1}cm^{-1}. The composition of the complex as determined by the continuous variation and mole ratio methods is 1 : 1 and the apparent stability constant of the complex at pH 2·5 and 27°C has the log K value of 4·2. The effects of buffer, time, reagent concentration and diverse ions are reported.

INTRODUCTION

PHENOTHIAZINE was proposed for the spectrophotometric determination of palladium (II)[1]. The authors have now developed phenothiazine as a sensitive reagent for the rapid spectrophotometric determination of platinum (IV). The proposed method offers the advantages of simplicity, rapidity and good sensitivity without the need for heating or extraction.

EXPERIMENTAL

Standard platinum (IV) solution was prepared by dissolving a known weight of platinum wire (99·99% pure) in hot aqua regia. The resultant solution was evaporated almost to dryness. A small amount of hydrochloric acid was added and the solution was again evaporated to dryness. The treatment was repeated three or four times in order to destroy any nitroso complexes. After the final evaporation, 5 ml of hydrochloric acid was added and the solution was made up to 500 ml with doubly distilled water. The stock solution was further diluted to give a solution of 10 μg/ml. A 0·1% solution of phenothiazine was prepared from recrystallized product in 80% ethyl alcohol. Buffer solutions in the pH range 0·5–5·0 were prepared employing 1 M sodium acetate and 1 M hydrochloric acid solutions. All other solutions were prepared from reagent grade chemicals. Beckman Model DB spectrophotometer was used for all absorbance measurements. ELICO pH meter was used for checking the pH of the solutions.

Procedure.—An aliquot of the stock solution containing 5 to 85 μg of platinum (IV) was transferred to a 25 ml volumetric flask. Sodium acetate-hydrochloric acid buffer (5 ml) and the requisite volume of ethanol to make the final volume of the solution 42% with respect to ethanol and 4 ml of 0·1% phenothiazine solution were added and the solution was diluted to 25 ml with doubly distilled water. The solution was mixed well and the absorbance was measured at 440 nm after standing 20 minutes, against a reagent blank prepared identically without platinum. The amount of platinum was then deduced from the standard calibration curve.

RESULTS AND DISCUSSION

Phenothiazine forms an yellowish-brown complex with platinum (IV) in sodium acetate-hydrochloric acid buffer at room temperature (27 ± 1° C). The optimum pH range for the complex formation is 0·5–3·5. A 24-fold molar excess of the reagent is required to obtain the maximum intensity of colour. The absorbance values remain constant over the range of ethanol concentrations 40–44% (V/V). Below 40% ethanol concentration, the reagent undergoes precipitation and above 44% the maximum intensity of the colour of the complex is not reached. The yellowish-brown complex exhibits absorption maximum at 436–442 nm. The absorption spectra of the reagent and platinum (IV) show that they do not absorb at this wavelength.

The platinum-phenothiazine complex is formed at room temperature and constant absorbance values are obtained only 20 minutes after adding the reagent to the platinum solution in sodium acetate-hydrochloric acid buffer medium containing ethanol. The absorbance values remain constant for one hour after complete complex formation. Beer's law is valid over the platinum concentration range 0·2–3·4 ppm. The optimum concentration range, for the effective spectrophotometric determination evaluated by Ringbom's method[2,3] is 1·0 to 3·0 ppm. According to Sandell's expression, the sensitivity of the reaction is 0·02 μg/cm^2 and the molar absorptivity is $9\cdot7 \times 10^3$ litre mole^{-1} cm^{-1}. Sample solutions containing 2 μg/ml of platinum (IV) prepared by the standard procedure gave a relative error of ± 0·8% and standard deviation of 0·002.

The following amounts (μg/ml) of diverse ions are found to give less than 2% error in the determination of 2 μg/ml of platinum (IV): rhodium (III) 20; iridium (III) 16; ruthenium (III) 4; osmium (VIII) 0·8; iron (III) 2; cobalt (II) 110; nickel (II) 25; copper (II) 2; sulphate 2500; nitrate 2200; phosphate 1200; chloride 5000; bromide 2000; acetate 2400 and oxalate 260. Palladium (II), gold (III), silver (I) and iodide interfere even in small amounts. Efforts to increase the tolerance limit of cations by the addition of masking reagents were unsuccessful.

Composition and stability constant of the complex.—The composition of the complex formed has been studied by Job's method of continuous variation[4,5] using equimolar solutions and by mole ratio method[6]. The determinations are made in a final volume of 25 ml and at pH 2·5 ± 0·1. The concentration of ethanol is maintained throughout the determinations at 42%. The absorbance of the solutions is measured at 440 nm. The results indicate the composition as 1 : 1. The observations at 410 and 470 nm also confirm the existence of only one complex. The stability constant values of the complex have been determined by two different methods: (*a*) Method of Foley and Anderson[7] modified by Dey and Coworkers[8] and (*b*) Mole ratio method[6]. The values of log K obtained for the platinum-phenothiazine complex at 27° C and at pH 2·5 ± 0·1 are 4·15 ± 0·1 and 4·25 ± 0·1 by the respective methods.

The nature of the complex has been studied by passing an aliquot of the solution of complex through the cation exchange resin, Amberlite IR-120 (H). The complete absorption of the colour of the complex by the ion exchanger has indicated that the complex is cationic.

The sensitivity of the proposed method is found to be more than that of tin (II) chloride[9], anthranilic acid, *o*-amino-phenol-*p*-sulphonic acid[10] and acenaphthenequinone monoxime[11] which have been proposed as sensitive spectrophotometric reagents for platinum (IV).

Determination of platinum in alloys.—Synthetic mixtures corresponding to platinum-rhodium thermocouple wire were prepared and the platinum content was determined following the standard procedure. The results are given in Table I.

TABLE I

Determination of platinum in synthetic mixtures corresponding to platinum-rhodium thermocouple wire

Pt (IV) taken, ppm	Rh (III) added, ppm	Pt (IV) found*, ppm
1·2	0·16	1·210
1·8	0·27	1·808
2·4	0·36	2·400
3·0	0·45	3·020

*The amount of platinum (IV) found is based on ten determinations.

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1. Sanke Gowda, H. and Keshavan, B., *Mikrochim. Acta*, 1975, 1, 437.
2. Ringbom, A., *Anal. Chem.*, 1938, 115, 332.
3. Ayres, G. H., *Ibid.*, 1949, 21, 652.
4. Job, P., *Ann. Chim.*, 1928, 9, 113.
5. Irving, H. and Pierce, T. B., *J. Chem. Soc.*, 1959, p. 2565.
6. Yoe, J. H. and Jones, A. L., *Ind. Eng. Chem. Analyt.* Ed., 1944, 16, 111.
7. Foley, R. T. and Anderson, R. C., *J. Am. Chem. Soc.*, 1948, 70, 1195; 1949, 71, 909.
8. Mukherji, A. K. and Dey, A. K., *Analyt. Chim. Acta*, 1958, 18, 324; *J. Inorg. Nucl. Chem.*, 1958, 6, 314.
9. Ayres, G. H. and Meyer, A. S., *Analyt. Chem.*, 1951, 23, 299.
10. Majumdar, A. K. and Sen Gupta, J. G., *Z. Analyt. Chem.*, 1960, 177, 265.
11. Sindhwani, S. K. and Singh, R. P., *Talanta*, 1973, 20 (2), 248.

SPECTRAL AND MAGNETIC PROPERTIES OF Co(II) COMPLEXES OF 5-PHENYLAZO-SALICYLALDEHYDES

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ABSTRACT

The Co(II) complexes of 5-phenylazo-salicylaldehydes are characterised using magnetic and spectral properties. The Co(II) complexes of 5-(phenylazo)-salicylaldehyde, 5-(*p*-chlorophenylazo)-salicylaldehyde, 5-(*o*-tolylazo)-salicylaldehyde show an octahedral environment while, Co(II) complexes of 5-(*p*-tolylazo)-salicylaldehyde, 5-(*o*-chloro phenylazo)-salicylaldehyde, 5-(*m*-chlorophenylazo)-salicylaldehyde are probably having distorted octahedral structure. The Co(II) complex of 5-(*m*-tolyl azo)-salicylaldehyde, however seems to be a planar one.

INTRODUCTION

THOUGH much work has been done on azo dye metal complexes, the ligand 5-aryl azo salicylaldehyde has attracted very little attention in the structural chemistry. A stray paper by Ohta *et al.*[1] mentions the preparation of Cu(II), Ni(II) and Co(II) complexes of 5-aryl azo salicylaldehyde but no useful discussion, as regards the configuration, has been given. Recently Mohan Das and Trivedi[2] carried out spectral analysis of U(IV) and Pd(II) complexes of *p*-phenyl azo salicylaldehyde. In this laboratory, Co(II) complexes of 5-(phenyl azo)-salicylaldehyde were studied mainly to throw light on the configuration of these complexes using spectral and magnetic data.

EXPERIMENTAL

Preparation of the Ligands and Their Metal Complexes

For the preparation of the ligands, the method reported earlier by Mohan Das and Trivedi[2] was used. Cobalt complexes of 5-(*o*-tolyl azo)-salicylaldehyde (*o*-TAS), 5-(*p*-tolylazo)-salicylaldehyde (*p*-TAS), 5-(*o*-chloro phenylazo)-salicylaldehyde (*o*-chloro PAS) and 5-(*m*-chloro phenylazo)-salcylaldehyde (*m*-chloro PAS) were prepared by adding an ethanolic solution of $CoCl_2.6H_2O$ (0·01 M) to the ligand in ethanol (0·02 M). The pH was adjusted to 5–6 by adding alcholic ammonia (2·5 M). The contents were stirred and the precipitate separated was washed, filtered and dried in a vacuum desiccator. Co(II) complex of 5-(phenylazo)-salicylaldehyde (PAS) was prepared by adding an alcoholic solution of cobalt acetate (0·01 M) to the ligand solution in ethanol (0·02 M). The contents were stirred overnight, the yellow coloured precipitate which separated out was filtered, washed and dried in a vacuum desicctor. Similarly, Co(II) complex of 5-(*p*-chloro-phenylazo)-salicylaldehyde (*p*-chloro PAS) was obtained by adding cobalt acetate (0·01 M) to the ligand (0·02 M) in toluene. Co(II) complex of 5-(*m*-tolyl azo)-salicylaldehyde (*m*-TAS) separated out as yellow complex when the ligand (0·02 M) and cobaltous nitrate (0·01 M) were refluxed in ethanol for 2 hours. The pH was adjusted to ~ 6.

The cobalt content of the complexes was estimated by EDTA titration using murexide as indicator and ammonium acetate-ammonia as buffer.

Physical measurements, such as diffuse reflectance spectra, infrared spectra, magnetic moments, were recorded on the instruments described previously[3].

RESULTS AND DISCUSSION

The elemental analyses given in Table I show clearly the metal to ligand ratios as 1 : 2 and presence of two molecules of water in each case. The IR data for the cobalt complexes showed the complete disappearance of OH bending frequency (1280–1272 cm^{-1}) and in plane deformation frequency (1200 cm^{-1}) in the ligand molecules and considerable shifting of C=O frequency from 1640–1660 cm^{-1} in the ligand molecule to 1606–1645 cm^{-1}, after complexation, showing coordination through phenolic O^- and –CHO in all the complexes studied. The strong bending mode of water reported at 1630–1600 cm^{-1}[4] does not seem to interfere with the assignment of C=O frequency (~ 1650 cm^{-1}) in the present case as the dehydrated complexes gave exactly similar bands, as in the original complexes. The diffuse reflectance spectra of Co(II) complexes of *o*-chloro PAS, *o*-TAS and *p*-TAS resemble octahedral cobalt complexes[5], ν_3 band being observed at ~ 20830 cm^{-1} and ν_1 at ~ 10200–10640 cm^{-1}. The ligand field parameters D*q* and B, calculated by using the expression[6].

$$Dq = \frac{2(\nu_1 + \nu_3) + (\nu_3^2 + \nu_1\nu_3 - \nu_1^2)^{\frac{1}{2}}}{20}$$

and B = $(\nu_3 - 2\nu_1) + 10\,Dq/15$, are 1118–1164 cm^{-1} and 718–762 cm^{-1} respectively. Though ν_3 frequency is not observed in cobalt(II) complexes of PAS, *m*-chloro PAS, *p*-chloro PAS, the absorption patterns for these are almost identical, the ν_3 is observed at 19230–20830 cm^{-1} and the

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* To whom all correspondence should be made.

TABLE I

Analytical data of Co(II) complexes

Co(II) complexes of	Co%	C%	H%	N%
PAS $(C_{26}H_{18}O_4N_4)2H_2O$	10·28 (10·02)*	57·2 (58·4)	4·1 (5·0)	10·3 (10·0)
o-Chloro PAS $(C_{26}H_{18}O_4N_4Cl_2)2H_2O$	9·60 (9·48)	50·9 (51·2)	3·2 (3·4)	9·1 (9·6)
m-Chloro PAS $(C_{26}H_{16}O_4N_4Cl_2)2H_2O$	9·60 (9·35)	50·8 (51·0)	3·2 (4·2)	9·1 (9·5)
p-Chloro PAS $(C_{26}H_{16}O_4N_4Cl_2)2H_2O$	9·60 (9·40)	50·8 (51·0)	3·2 (4·0)	9·1 (9·4)
o-TAS $(C_{28}H_{22}O_4N_4)2H_2O$	10·28 (10·63)	58·6 (58·4)	4·5 (5·0)	9·8 (9·8)
m-TAS $(C_{28}H_{22}O_4N_4)2H_2O$	10·28 (10·11)	58·6 (58·3)	4·5 (5·1)	9·8 (9·7)
p-TAS $(C_{28}H_{22}O_4N_4)2H_2O$	10·28 (10·00)	58·6 (58·7)	4·5 (4·8)	9·8 (9·6)

* Values found are given in paranthesis.

TABLE II

Room temperature magnetic data of Co (II) complexes

Co(II) complexes of	$\chi_H \times 10^6$ c.g.s.u.	$\chi_M' \times 10^6$ c.g.s.u.	μ_{eff} B.M.	T° K
PAS	11910·7	12148·7	5·39	299
o-Chloro PAS	7696·4	7974·4	4·37	299
m-Chloro PAS	7048·4	7325·4	4·19	300
p-Chloro PAS	9790·4	10068·5	4·92	298
o-TAS	12873·7	12241·7	5·42	298
m-TAS	1675·1	1943·1	2·16	300
p-TAS	7484·3	7752·3	4·31	299

other bands being ~ 23000, 16670 cm^{-1}. Cobalt complex of *m*-TAS showed the absence of the characteristic band ~ 20830 cm^{-1} which is typical of octahedral cobalt(II) system[7].

The magnetic moments (Table II) of the cobalt complexes of PAS, *p*-chloro PAS and *o*-TAS lie in the range generally expected for the octahedral environment, *i.e.*, 4·7–5·42 B.M. However, the room temperature magnetic moments of cobalt(II) complexex of *o*-chloro PAS, *m*-chloro PAS and *p*-TAS are in the range 4·19–4·37 B.M, lower than the moments observed for octahedral cobalt(II) and tetrahedral cobalt(II) complexes[8]. Since tetrahedral geometry seems to be less likely for the complexes, as seen from diffuse reflectance spectra, and, as Co(II) complexes having magnetic moments as low as 4·2 B.M.[9] have been reported for octahedral symmetry, it seems that the Co(II) complexes of *o*-chloro PAS, *m*-chloro PAS and *p*-PAS are probably octahedral. Lowering of the symmetry will result in splitting up of the ground term state and hence the orbital contribution will be quenched thus resulting in low magnetic moments[10]. Co(II) complex of *m*-TAS, however, registers a magnetic moment of 2·16 B.M. Though the planar Co(II) complexes of phthalocyanine, ethylene-diimine *bis*-salicylaldehyde, *o*-phenylene *bis*-dimenthylarisine have been found to have room temperature magnetic moments 2·10–2·72 B.M[11], it is known that the Co(II) planar complexes have not been well characterised. A few papers dealing with spectral studies of planar Co(II) complexes have been published[12]. As the spectra at ~ 8300 cm^{-1} was not taken, no definite conclusion could be drawn about Co(II) complex of *m*-TAS.

1. Ohta, H., Nawata, K.; Soejima; C. and Tsumakt, T., *Mem. Fac. Sci. Kyushu University Serv.*, 1962, C 5, 1.
2. Mohan Das, P. N. and Trivedi, C. P., *J. Ind. Chem. Soc.*, 1972, 49, 739.
3. Deshmukh, K. G. and Bhobe, R. A., Communicated to *J. Inorg. Nucl. Chem.*
4. Nakamoto, K., *I.R. Spectra of Inorg. and Coordinate Compounds*, New York, John Wiley and Sons, Inc., 1963, p. 156.
5. Carlin, R. L., *Transition Metal Chemistry*, 1965, 1, 1.
6. Eibeck, W. J., Holmes, F. and Underhill, A. E., *J. Chem. Soc. (A)*, 1967, p. 757.
7. Cotton, F. A. and Holm, R. H., *J. Am. Chem. Soc.*, 1960, 82, 2983.
8. -- and —, *Ibid.*, 1960, 82, 2979.
9. Singh, B., Lakshami, and Aggarwal, U., *Inorg. Chem.*, 1969, 8, 2341.
10. Earnshaw, A., *Introduction to Magnetochemistry*, Acad. Press, London and New York, 1968, p. 43.
11. Figis, B. N. and Nyholm, R. S., *J. Chem. Soc.*, 1959, p. 338.
12. Nishikawa, N. and Yamada, S., *Bull. Chem. Soc. Japan*, 1964, 37, 8; Gray, H. B., Williams, R., Bernal, I. and Billig, E., *J. Am. Chem. Soc.*, 1962, 34, 3596; Busetto, C., Cariati, F., Fusi, A., Culloti, M., Morazzo, F., Passini, A., Ugo, R. and Valenti, V.; *J. Chem. Soc. Dalton*, 1973, p. 754; Weigold, H. and West, B. O., *J. Chem. Soc. (A)*, 1967, p. 1310.

STUDIES ON THE COPPER COMPLEX OF ISONICOTINIC ACID HYDRAZIDE—A NEW ANTIVIRAL DRUG

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ABSTRACT

Detailed studies have been carried out to elucidate the precise nature of the complex formed between copper and isoniazid as this complex is now shown to be active against animal viruses. IR and ESR data provide definite proof for the existence of both cupric and cuprous complexes with isoniazid. A possible structure of cuprous-isoniazid complex is proposed.

1. INTRODUCTION

ISONICOTINIC acid hydrazide (isoniazid) is one of the potent drugs used in the treatment of tuberculosis. The bactericidal action of isoniazid is well understood[1]. Cupric ions enhance the activity of isoniazid *in vitro* against *Mycobacterium tuberculosis*[2-4]. Cupric ions form 1 : 1 (monodentate) and 1 : 2 (bidentate) complexes (chelates) with isoniazid[5-7]. Rieber and Bemski[7] reported that under aerobic conditions, not more than 20% of Cu^{2+} is reduced during the complex formation with isoniazid, whereas the report of Krivis and Rabb[8] showed that approximately 92% of the Cu^{2+}, which is precipitated as copper-isoniazid complex, exists in cuprous form.

In the light of the above conflicting reports, the present study was undertaken to determine the precise nature of the copper-isoniazid complex, as this drug is a strong inhibitor of the *in vitro* reverse transcriptase activity of Rous sarcoma virus and is now shown to be active against both DNA and RNA animal viruses[9,10]. In this report, we present data to show the formation of two different complexes between copper and isoniazid, depending upon the oxidation state of the metal ion.

2. MATERIALS AND METHODS

Isoniazid (Sigma Chemical Co., USA), vanillin (B.D.H.) and other reagents used were of analytical grade.

2.1. *Preparation of the Copper-Isoniazid Complexes*

Cupric and cuprous complexes were prepared separately by mixing equal volumes of the equimolar solutions[7,8]. For cuprous-isoniazid complex, freshly prepared cuprous chloride was used. The precipitate obtained in each case was washed extensively with distilled water, dried and stored at 4° C.

2.2. *Paper Chromatography*

Ascending paper chromatography was done using Whatman No. 1 paper with isopropanol : glacial acetic acid : water (12 : 3 : 5 ; v/v/v) as the solvent system.

2.3. *Estimation of Isoniazid*

Isoniazid was estimated by the method of Kelly and Poet[11]. The yellow colour, developed by the addition of 0·3 ml of 2% vanillin solution in 1 N NaOH to 3 ml of 1 N H_2SO_4 containing either isoniazid or copper-isoniazid complexes was measured at 380 nm.

2.4. *Reverse Transcriptase Assay*

15 μl of purified SR strain of Rous sarcoma virus (suspended in 100 μl of a solution containing 8 mM Tris buffer of pH 8·1, 8 mM $MgCl_2$, 200 μM each of dATP, dCTP and dGTP), was exposed to 40 μM isoniazid, cuprous-isoniazid or cupric-isoniazid complex separately and incubated at 37° C for 15 min. Five μl of 1% NP–40 and 10 μl of ^{3}H-TTP (10 μCi/ml, Sp. Act. 23·9 Ci/mmol) were then added. The reaction mixture was incubated at 37° C for 60 min. and after the addition of 0·5 ml of 2% sodium pyrophosphate and 2 ml of 10% TCA, the precipitate was filtered, washed, dried and the radioactivity was determined in a Beckman LS-100 liquid scintillation spectrometer.

2.5. *IR Spectrophotometry and ESR Spectrometry*

IR spectra of isoniazid, cuprous-isoniazid and cupric-isoniazid complexes were recorded in UR 10 Carl Zeiss Jena double beam IR spectrophotometer at 25° C. The KBr pellet technique was followed as described by Tu and Reinosa[12].

ESR spectra of both the complexes were recorded in an ESR spectrometer at about 9,300 MHz with 100 KHz modulations. All recordings were carried out at 25° C. A marker of $g = 2 \cdot 0036$ was used to calibrate the magnetic field.

3. Results and Discussion

A few characteristic properties of the two complexes are given in Table I. The precipitation of the cupric-isoniazid complex takes a little more time than that of the cuprous-isoniazid complex, indicating that initially, 1 : 1 (monodentate) complex is formed between Cu^{2+} ions and isoniazid which later form 1 : 2 (bidentate) complex[7]. On the other hand, Cu^{+} ions and isoniazid form only 1 : 1 (monodentate) complex. This was proved by estimating the amount of bound isoniazid present in both the complexes. The amount of isoniazid in the cupric-isoniazid complex was twice that present in the same concentration of the cuprous-isoniazid complex (Table I). Microanalysis of both the complexes further proved this point. (Data not shown).

TABLE I
Characteristic properties of the two complexes formed between copper and isoniazid

	Cuprous-isoniazid	Cupric-isoniazid
1. Precipitation time	5–7 min	10–12 min.
2. Colour and nature	Yellow, thin and powdery	Green, clumped and flocculent
3. R_f value	0·73	0·64
4. Stoichiometry	1 : 1 (Monodentate)	1 : 2 (Bidentate)
5. Melting point	~250° C d.	~280° C d.
6. Reverse transcriptase inhibition (40 μM)	45%	95%

The chemical structures of the complexes and the involvement of the groups of isoniazid side chain in the complex formation were ascertained by IR spectrophotometry. The IR spectra of isoniazid, cuprous-isoniazid and cupric-isoniazid complexes are presented in Fig. 1. The absorption between 1650–1680 cm^{-1} in the IR spectrum of cuprous-isoniazid complex indicates the presence of $-C{=}O$ group of $-CO.NH.NH_2$, and the hydrogen bonded $-NH$ group of isoniazid is indicated by the absorption in the region 2950–3200 cm^{-1}. The absence of the sharp absorption peak at 3300 cm^{-1} by $-NH_2$ group indicates the involvement of $-NH_2$ group in the

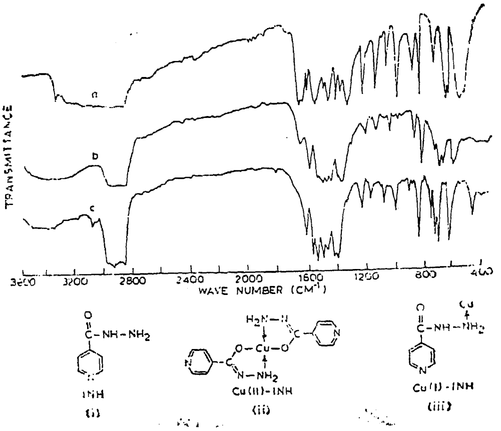

FIG. 1. IR spectra of isoniazid (*a*), cuprous-isoniazid complex (*b*), and cupric-isoniazid complex (*c*). A possible structure of cuprous-isoniazid complex is shown in (*iii*). Structure (*ii*) is redrawn from Fallab and Erlenmayer[13]. (*i*) INH-isonicotinic acid hydrazide.

complex formation. The IR spectrum of cupric-isoniazid complex indicates the presence of only –NH group. Both $-NH_2$ and –C=O groups of isoniazid side chain are involved in the complex formation, since the corresponding peaks disappear from the cupric-isoniazid complex spectrum[12,13]. The presence of intact pyridine nucleus of isoniazid by the aromatic C=C stretching vibrations at 1580 cm^{-1} in both the cases indicates the non-involvement of the pyridine nucleus in the complex formation.

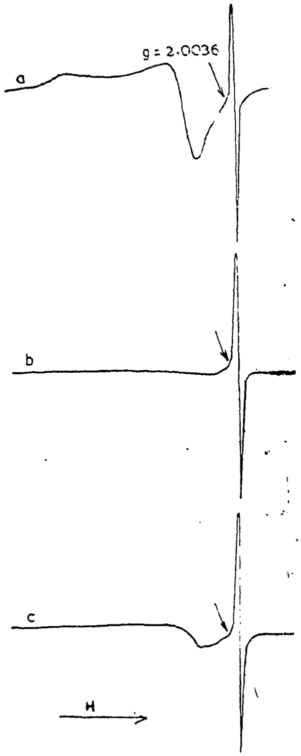

FIG. 2. ESR spectra of cupric-isoniazid complex (*a*), cuprous-isoniazid complex (*b*), and oxidized cuprous-isoniazid complex (*c*).

The ESR spectra of the complexes (Fig. 2) rules out the possibility of any significant reduction of Cu^{2+} to Cu^{+} during the complex formation as proposed by Krivis and Rabb[8]. The cupric-isoniazid complex gave an absorption around 3200 G with a g value of 2·30 (as was obtained by Rieber and Bemski[7]), the complex showing less detail and a slight shift towards the lower magnetic field as compared with the best resolved hyperfine structure, with four lines superposed on the large absorption by cupric ions alone. On the other hand, under similar conditions, cuprous-isoniazid complex did not show any absorption. However, a very weak signal was obtained when cuprous-isoniazid complex was subjected to oxidizing conditions. These results show that there is no significant reduction of Cu^{2+} to Cu^{+} during the complex formation, and Cu^{+} ions themselves are capable of forming another complex with isoniazid. Oxidation of Cu^{+} to Cu^{2+} may occur under aerobic conditions to an extent of not more than 5%, since isoniazid serves as an antioxidant.

The present results, therefore, support the finding of Rieber and Bemski[7], that the complex formation between cupric ions and isoniazid does not involve reduction of cupric ions and a 1 : 2 (bidentate) complex is formed.

The inhibition of the *in vitro* reverse transcriptase activity of Rous sarcoma virus and also the transforming ability of the virus in chick embryo fibroblast cell culture by the cupric-isoniazid complex, is significantly more than either isoniazid or cuprous-isoniazid complex[9,10]. Furthermore, cupric-isoniazid complex is least toxic to the animal cells (primary chick embryo fibroblast and vero cells) grown in stationary cultures, as compared with either isoniazid or cuprous-isoniazid complex (Unpublished results). A detailed study on the nature of the physiologically active molecular complex is now under investigation.

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1. Krishna Murthy, C. R., *Antibiotics*, Corcoran, J. W. and Hahn, F. E., eds., Springer-Verlag, Berlin. 1974, 3, 623.
2. Fallab, S. and Erlenmayer, H., *Helv. Chim. Acta*, 1953, 36, 6.
3. Winder, F., *Amer. Rev. Resp. Dis.*, 1960, 81, 69.
4. Cymerman-Craig, J., Willis, D., Rubbo, S. D. and Edgar, J., *Nature*, 1955, 176, 34.
5. Bjerrum, J. B. and Balhausen, C. J., *Acta Chem. Scand.*, 1954, 8, 1275.
6. Kivelson, D. and Neiman, R., *J. Chem. Phys.*, 1961, 35, 140.
7. Rieber, M. and Bemski, G., *Arch. Biochem. Biophys.*, 1969, 131, 655.
8. Krivis, A. F. and Rabb, J. M., *Science*, 1969, 164, 1064.
9. Antony, A., Arun Srivastava, Ramakrishnan, T., Nandi, U. S. and Levinson, W. E., *Proc. Assoc. Microbiol.*, India, 1974, 15, 9.
10. —, —, —, —, Mikelens, P., Jackson, J. and Levinson, W. E., *IUB Symposium on the Molecular Basis of Host-Virus Interaction*, Varanasi, 1976, p. 61.
11. Kelly, J. M. and Poet, R. B., *Amer. Rev. Tuber. Pulm. Dis.*, 1952, 65, 484.
12. Tu, A. T. and Reinosa, J. A., *Biochemistry*, 1966, 5, 3375.
13. Fallab, S. and Erlenmayer. H., *Experientia*, 1952, 8, 298.

LETTERS TO THE EDITOR

MEAN SQUARE AMPLITUDES FOR THE IN-PLANE VIBRATIONS IN ACETANILIDE AND DEUTERATED ACETANILIDE

THE authors in their earlier investigation have carried out the normal co-ordinate treatment of acetanilide and N-Deuterated acetanilide[1] using the general quadratic potential function as a force field. The calculation of potential energy distribution of each normal mode among various symmetry co-ordinates has enabled the authors to discuss the nature of the Amide I, Amide II and Amide III bands in acetanilide and N-Deuterated acetanilide. These results have indicated that the Amide I band is due to (C=O) stretch and the Amide II and Amide III bands are due to the combined contributions of δ (N–H) and ν (C–N) modes of vibrations as in the case of N-Methyl acetamide[2].

In the present note, we report the mean square amplitudes and the mean amplitudes of vibration of the various linkages of the acetanilide and N-Deuterated acetanilide using the 'L' matrix method. The mean square amplitudes of vibration is given by L Δ $\tilde{L}$, where $\tilde{L}$ is the transpose of the L matrix and Δ is the diagonal matrix with elements

$$\Delta_k = \langle Q_k^2\rangle = \frac{h}{8\pi^2\nu_k}\coth\left(\frac{h\nu_k}{2kT}\right)$$

Where $\langle Q_k^2\rangle$ is the mean square of the kth normal co-ordinate. The mean square amplitudes and the mean amplitudes at temperatures 0° K and 300° K are given in Table I.

TABLE I

Mean square amplitudes and mean amplitudes of various linkages in acetanilide and N-Deuterated acetanilide

Bond and mode of vibration	Molecule	Mean square amplitudes (in Å²) at		Mean amplitudes (in Å) at	
		T = 0° K	T = 300° K	T = 0° K	T = 300° K
ν(C–N)	Acetanilide	0·001872	0·001914	0·0433	0·0438
	Deuterated Acetanilide	0·001853	0·001876	0·0430	0·0433
ν(C=O)	Acetanilide	0·001636	0·001650	0·0404	0·0406
	Deuterated Acetanilide	0·001639	0·001652	0·0405	0·0410
ν(C–CH$_3$)	Acetanilide	0·002522	0·002635	0·0502	0·0513
	Deuterated Acetanilide	0·002525	0·002622	0·0504	0·0512
ν(N–C$_6$H$_5$)	Acetanilide	0·001626	0·001773	0·0403	0·0421
	Deuterated Acetanilide	0·001613	0·001734	0·0401	0·0416

The mean amplitudes of vibration of ν (C= and ν (C–N) in acetanilide and N-Deuterat acetanilide are lower than that of the same linka in N-Methyl acetamide[2]. The variation of the m amplitude of vibration with temperature is obser to be small in all these linkages.

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---

1. Venkata Chalapathi, V. and Venkata Ran K., *Proc. Indian Acad. Sci.*, 1968, 67,
2. — and —, *Ibid.*, 1966, 64, 148.

A COMPARISON OF THE BANDS IN THE LABORATORY EMISSION SPECTRUM OF *m*-HYDROXYBENZALDEHYDE AND THE CO^+ BANDS IN COMET TAIL SPECTRA

A GREAT deal of experimental work has been devoted to the study of emission spectra of benzaldehyde and its derivatives[1-4]. The present note reports an unusual band system due to *m*-hydroxybenzaldehyde. The spectrum was photographed on a Q-24 medium quartz and a Fuess glass spectrograph using* the experimental procedure described in earlier works[1-4]. Figure 1 shows the

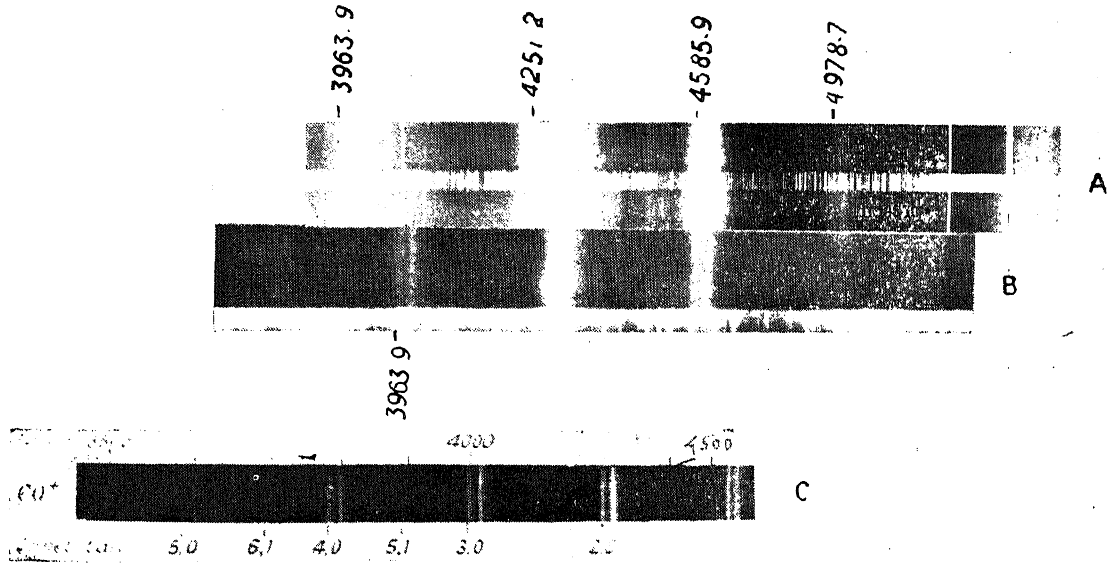

FIG. 1. Emission spectrum of *m*-OH benzaldehyde. A : in Fuss glass spectrograph ; B : in Medium quartz spectrograph and C : Comet tail band taken from reference 5.

TABLE I

Bands observed in m-OH benzaldehyde and the bands in the tail CO system

Tentative assignment	Present system Wavenumber cm⁻¹	Wavelength Å	Intensity	Comet tail system Wavelength Å	Intensity	Assignment
0–0	26895	3707·1	vw	3707·4	9	Q_1
		3718·2		3724·9		Q_2
0–1680	25227	3963·9	vs	3957·0	7	P_2
		3989·1		3977·7	4	P_1
0–2 × 1680	23524	4251·2	vs	4252·4	..	Q_2
		4280·2		4274·3	10	Q_1
		4485·9		..		
		4505·2	vw	4518	3	R_1
0–3 × 1680	21805	4585·9		4565·8		R_2
		4625·4	s	4539·0	8	R_1
		4978·7		5039·7		R_1
		5018·4	s	5072·1	5	R_2
		5437·3		5461·4		R_1
		5473·0	vw	5499·9	6	R_2
		5960·0		5970		R_2
		5998·2	w	6015	0	R_1

s = strong, vs = very strong, w = weak and vw = very weak.

emission spectrum of the molecule. The spectrum of the comet tail band CO^+ is given for a direct comparison.

The conspicuous feature of the spectrum is its striking similarity with the comet tail band system due to CO^+ [5,6,7]. It does not bear any resemblance with the emission spectra of benzaldehyde or its derivatives. Measurements however show that the present band system does not coincide with the comet tail system or the Johnson–Baldet system[6]. Like the comet tail system the present system also consists of double headed bands but the bands are more diffuse. For comparison the bands in the comet tail system of CO^+ can be correlated with the bands of the present system. This is shown in Table I. The present system appears to be a CO^+ system shifted to the lower energy side.

It may be noted that the present emission spectrum is tentatively analysed on the basis of a 0, 0 band at 3718 Å with a carbonyl stretching band (ν = 1680 cm^{-1}) superposed on it. This type of analysis has been made on the emission spectra of identical molecules. However, from the striking similarity between the bands of CO^+ and the present band system we infer that the present band system may not actually belong to the molecule under investigation but corresponds to some intermediate molecule or radical formed as a result of dissociation, occurring in the process of discharge. It may further be added that there is also a similarity in the process of excitation of the two systems. The comet tail system in the laboratory was observed in the discharge tube containing helium with a trace of CO while the present system was observed in the discharge tube containing *m*-hydroxybenzaldehyde with flowing vapour of benzene.

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Dibrugarh 786 004, *June* 22, 1976.

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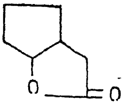
* The experiment was performed at the spectroscopy laboratory of B.H.U., Varanasi.

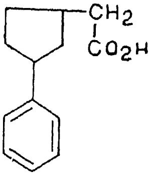
1. Robinson, G. W., *J. Chem. Phys.*, 1954, 22, 1384.
2. Garg, S. N. and Singh, N. L., *Sci. Res. J.*, B.H.U., 1958, 9, 61.
3. Juyal, D. P., *Curr. Sci.*, 1965, 34, 175.
4. Singh, N. L. and Juyal, D. P., *Indian J. Pure and Appl. Phys.*, 1965, 3, 342.
5. Pearse, R. W. B. and Gaydon, A. G., *Identification of Molecular Spectra*, Chapman and Hall. Ltd., 1963, p. 121.
6. Rao, K. N., *Astrophys. J.*, 1950, 3, 306.
7. Johnson, R. C., *P.R.S.*, 1905, 108, 343.

FRIEDEL-CRAFTS ALKYLATION OF AROMATIC HYDROCARBONS WITH THE LACTONE OF 2-HYDROXYCYCLOPENTANEACETIC ACID

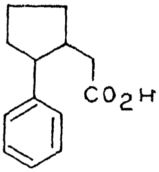
THE $AlCl_3$-catalysed alkylation of aromatic hydrocarbons with simple unsubstituted alicyclic lactones like the lactone of trans 2-hydroxycyclohexaneacetic acid was studied by Phillips and Chatterjee[1] who observed that the reaction was attended with extensive rearrangement leading to the formation of a small amount of the expected trans 2-arylcyclohexaneacetic acid together with a large amount of the rearranged product consisting of trans 4-arylcyclohexaneacetic acid and the 3-aryl isomer of unknown stereochemistry. The reaction was interpreted as going through the carbonium ion formed by the action of the Lewis acid on the lactone followed by a series of 1, 2 shifts and electrophilic attack on the arenes. The observation by Friedman[2] that the alkylation of aromatics by branched chain cycloolefines like methyl cyclohexene in the presence of Lewis acid catalyst leads to the formation of a large amount of the secondary alkylate but very little of the tertiary alkylate led Chatterjee and Bhattacharyya[3] to investigate the cationic nuclear alkylation of aromatics with the lactone of 4-methyl-2-hydroxycyclohexaneacetic acid. Those investigators isolated 2-aryl- and 3-arylcyclohexaneacetic acids to the exclusion of the 4-aryl isomer, the former components being successfully converted to polycyclic compounds by way of cyclisation, reduction and dehydrogenation. The facile rearrangement in the cyclohexane system is quite common and in order to see if similar rearrangement of the carbonium ion in the cyclopentane system can also take place, the lactone of cyclopentanol-2-acetic acid (I) has been prepared and the $AlCl_3$-catalysed alkylation of benzene with this lactone has been investigated.



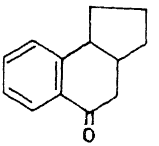
I



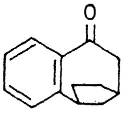
II



III



IV



V

The lactone (I) has been prepared from 2-carbethoxycyclopentanone by condensing the *sodio* derivative with ethyl chloroacetate followed by hydrolysis

The resulting cyclopentanone-2-acetic acid, m.p., 53°, obtained in 80% yield was esterified and reduced by aluminium isopropoxide in isopropanol to give the hydroxy acid which on short refluxing under acidic condition yielded the desired lactone (I), b.p. 98–100°/**1 mm**[4].

The catalysed condensation of the lactone (I) with benzene gave a stereoisomeric mixture of phenylcyclopentylacetic acids isolated through the ethyl ester, b.p. 145–148° at 1 mm in 69% yield. Fractional crystallisation from pet. ether led to the separation of pure cis 3-phenylcyclopentylacetic acid (II), m.p. 67°, amide, m.p. 113° and a stereoisomeric mixture of the 2-phenyl isomer (III), b.p. 146–147°/0·2 mm consisting predominantly of the trans variety (50%) which on short cyclisation by PPA yielded trans 1-oxo-1, 2, 3, 4-tetrahydro-3, 4-cyclopentenonaphthalene (IV), m.p. 90°, 2, 4-DNP derivative, m.p. 243°, the structure of which followed from its reduction and dehydrogenation to 1, 2-cyclopentenonaphthalene, b.p. 115–116°/1 mm, picrate, m.p. 105°[5]. The structure of the cis acid (II) was confirmed by its cyclisation to a known benzosuberone derivative (V), b.p. 135–140°/1 mm, 2, 4-DNP derivative, m.p. 210°[6] as well as comparison with an authentic specimen of (II) synthesised from ethyl cyclopentanone-3-acetate prepared from cyclopentadiene by addition of HBr followed by oxidation[7], and phenyl magnesium bromide. The resulting product on dehydration and hydrolysis gave 3-phenylcyclopent-2-ene-1-acetic acid (VI), m.p. 96° which on hydrogenation yielded the cis acid (II). No reduction of the lactone (I) to cyclopentyl acetic acid by the hydride transfer to the carbonium ion[3] was observed.

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1. Phillips, D. D. and Chatterjee, D. N., *J. Am. Chem. Soc.*, 1958. 80, 1360, 1911.
2. Friedman, B. S. and Morritz, F. L., *Ibid*, 1957, **79**, 1465.
3. Chatterjee, D. N. and Bhattacharyya, S. P., *Tetrahedron*, 1971, **27**, 4153; *Indian J. Chem.*, 1974, **12**, 958.
4. Linstead, R. P. and Meade, A. E., *J. Chem. Soc.* 1954, p. 935.
5. Synder, R. H. *et al.*, *J. Am. Chem. Soc.*, 1959, **81**, 4299.
6. Winternitz, F., Mousseron, M. and Rouzier, G., *Bull. Soc. Chim.*, 1953, p. 190.
7. Meinwald, J. and Frauenglass, E., *J. Am. Chem. Soc.*, 1960, **82**, 5235.

THE ULTRAVIOLET IRRADIATION OF S-PHENYL THIOBENZOATE

The photolysis of S-(*p*-tolyl) thiolacetate in cyclohexane using 254 nm light was reported by Grunwell[1] to give 77% of di-(*p*-tolyl) disulphide and 7% of *p*-tolyl methyl sulphide. No photo-Fries products were observed and explanations were offered for their absence. Loveridge *et al.*[2] investigated the u.v. irradiation of S-phenyl thiolacetate in benzene, and obtained, among other products, low yields (4% each) of the two photo-Fries products, the 2- and 4-acetyl thiophenols. Irradiation in other solvents such as ether, THF or cyclohexane gave still lower yields of the photo-Fries products; and no reaction was observed in methyl carbitol or ethanol. Since thiolbenzoates have not been investigated, it was of interest to study the u.v. irradiation of S-phenyl thiolbenzoate.

S-Phenyl thiolbenzoate (1·07 g, 0·01 mole) was dissolved in absolute ethanol (500 ml) and irradiated with a medium pressure u.v. lamp, under nitrogen atmosphere. A solid began to separate after about an hour and deposited on the walls of the irradiation well. Irradiation was continued with periodic removal of the solid from the well, till the starting material was completely photolysed (4 hr) as verified by TLC. After irradiation, the insoluble residue, a pale yellow amorphous powder, was filtered off (420 mg). This solid gave a positive test for organic sulphur, melted at the range 80–110° C and appeared to be polymeric in nature (IR: broad peaks at 1660, 1585, 1285, 745 and 700 cm^{-1}; NMR: broad multiplet centered at 7·28 δ due to aromatic protons). Similar polymer deposition was also observed in the irradiation of S-phenyl thiolacetate[2].

The filtrate on evaporation (reduced pressure) deposited diphenyldisulphide as a crystalline solid (180 mg; 17%: m.p. 61° C). The residue on chromatography on silica yielded (*a*) diphenyl thioether (120 mg.; 13%; b.p. 292° C) and (*b*) the photo-Fries product, 4-benzoyl thiophenol (110 mg.; 10·3%), m.p., 70–71° C. (lit.[3] 71–72° C); IR: 3070, 2580 (SH), 1685 (C = 0), 905 (*p*-disubstituted benzene), 740 and 690 cm^{-1} (monosubstituted benzene); NMR: singlet at 3·2 δ (1, SH) and multiplet centered at 7·18 δ (9 aromatic H).

The present study with S-phenyl thiolbenzoate gave only the *p*-isomer unlike in the irradiation of S-phenyl thiolacetate which gave both *o*- and *p*-isomers. This may be due to the larger bulk of the benzoyl radical. The absence of thiophenol among the reaction products, observed in the present study and also in the reported photolysis of S-(*p*-tolyl) thiolacetate[1], does not suggest that thiophenol is not formed during irradiation. In these studies, no attempts were made during the workup of the reaction mixture, to prevent atmospheric oxidation, and any thiophenol formed would have been oxidised to the disulphide. The presence of polymeric products in the present study would also suggest the formation of thiophenol[4].

Our thanks are due to the Director, Indian Institute of Science, Bangalore, for providing laboratory facilities for short-term work to one of us (R. R. P.) and the University of Calicut for financial support.

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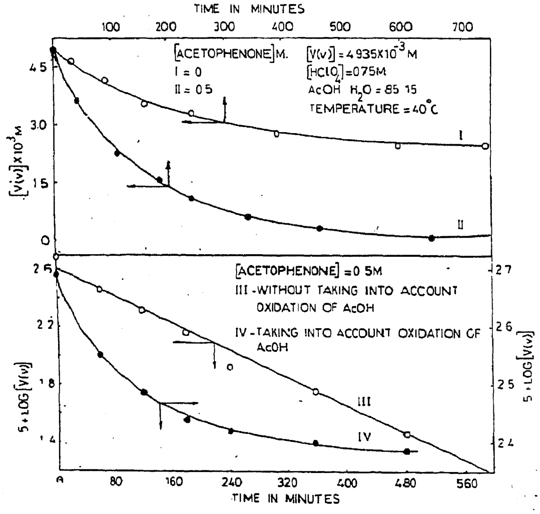
Kerala 673 635, *April* 19, 1976.

---

1. Grunwell, J. R., *J. Chem. Soc. (Chem. Comm.)*, 1969, p. 1437.
2. Loveridge, E. L., Beck, B. R. and Bradshaw, J. S., *J. Org. Chem.*, 1971, 36, 221.
3. Walker, D. and Leib, J., *Ibid.*, 1963, 28, 3077.
4. Schaafsma, Y., Bickel, A. F. and Kooyman, E. C., *Tetrahedron*, 1960, **10**, 76.

ON THE OXIDATION OF ACETOPHENONE BY VANADIUM (V) IN AQUEOUS ACETIC ACID

A SEARCH of the literature on the oxidation of organic substrates using vanadium(V) shows that many such oxidations are carried out in the presence of acetic acid[1-4]. Our investigations show that acetic acid purified by the procedure suggested by Orton and Bradfield[5] (freezing point 16·6° C) reacted with vanadium(V) giving carbon dioxide as one of the products. Hence oxidation of organic substrates by vanadium(V) in the presence of acetic acid has to be scrutinized carefully.



CONCENTRATION-TIME CURVES AND FIRST ORDER PLOTS FOR VANADIUM(V)

FIG. 1

Misra *et al.*[6] have reported a good first order, with respect to vanadium(V) for the oxidation of acetophenone by vanadium(V) in 85% acetic acid. They have not considered the possibility of a reaction of vanadium(V) with the medium. We have observed that in the oxidation of acetophenone by vanadium(V) ($4 \cdot 95 \times 10^{-3}$ M) in 85% acetic acid about 50% of the vanadium(V) was used u in the reaction with the solvent itself.

Since the reaction of the solvent can be followed independently, it is possible to separate the oxidation of acetophenone from the reaction with the solvent when both reactions are taking plac simultaneously. If we ignore the reaction of th solvent with vanadium(V) a neat first order wit respect to vanadium(V) is obtained. If w consider the reaction with the solvent also, th results do not fit into a first order plot (Fig. 1).

The intermediates formed in the reaction wit the solvent may interfere with the oxidation of th substrate, making the study of the substrate oxid tion in presence of acetic acid complex. Furthe work is in progress to understand the reaction vanadium(V) with acetic acid.

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Madras 600 036, *June* 26, 1976. J. RAJARAM.

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1. Radhakrishnamurthy, P. S. and Pati, S. C *J. Indian Chem. Soc.*, 1968, 45, 1012 ; *Phys. Chem.* (Leipzig), 1969, 241, 49.
2. Rama Shankar and Swami, S. N., *J. Indi Chem. Soc.*, 1963, 40, 105.
3. Manhot, U. S., Shankar, R. and Swami, S. N *Z. Phys. Chem.* (Leipzig), 1963, 222, 24
4. Rama Shankar and Joshi, S. N., *Ind. J. Che* 1963, 1, 289.
5. Orton, K. J. P. and Bradfield, A. E., *J. Che Soc.*, 1927, p. 983.
6. Misra, G. C., Sinha B. K. and Behera, G. *J. Indian Chem. Soc.*, 1975, L. 11, 1053.

DIGESTIVE ENZYMES OF PANCREAS IN NORMAL AND THIAMINE DEFICIENT RATS

ANOREXIA is one of the prominent sympto associated with thiamine deficiency[1-3]. Whether is due to a defect in the production and secreti of digestive enzymes is not clear. In the pres communication the amylase, lipase and proteoly activities of pancreas of normal and thiam deficient rats have been determined and report

Thiamine deficient male albino rats[4] along w their normal controls were sacrificed in the fed st pancreatic tissue cut out, weighed quickly and hor genized in saline or buffer using Potter-Elvehj homogenizer chilled in ice. For proteolytic activ the homogenization was carried out in 0·01 M (hydroxymethyl aminomethane)-0·03 M Ca buffer pH 7·5 for 5 min. at 0–4° C and the extent of digestion of casein in 30 min. at 37° C and pH 7·5 was estimated by the method of Kunitz[5]. Enterokinase was added to the homogenate and incubated for 1 hr for activation of the zymogen, before the addition of casein substrate. The reaction was stopped by addition of 10% trichloracetic acid and the tyrosine liberated estimated in the filtrate. The proteolytic activity is expressed as μ moles of tyrosine liberated/hr.

For amylase activity, the tissue was homogenized in 0·01 M KH_2PO_4–0·85% (w/v) NaCl buffer pH 6·5 and centrifuged at 10,000 g at 0° C in a MSE Mitral 2L refrigerated centrifuge and the supernatant fraction used. The digestion of starch at 37° C was measured as maltose after incubation forr 10 min. and precipitation by 0·3 N $Ba(OH)_2$ and 5% $ZnSO_4$ using the methods of Somogyi[6] and Nelson[7]. The amylase activity is expressed as that amount of enzyme which librates 1 mg of maltose/10 min.

Lipase activity was determined by using homogenates in 0·85% (w/v) NaCl by the method of Clarke[8] using olive oil-bile-glycerol mixture in 0·05 M NH_4Cl—ammonia buffer pH 8·0 containing $CaCl_2$. The lipase activity is expressed as ml of N/20 KOH required for tritration of fatty acids liberated/4 hr.

The nitrogen content was estimated by the micro-kjeldahl method[9].

When expressed per g fresh weight of pancreas or per mg tissue nitrogen, both amylase and proteolytic activities were elevated ($p < 0·01$ and $< 0·02$) in thiamine deficient pancreas, while lipase activity appeared unaffected. However, when the enzyme activity of the whole pancreas was considered, the lipase activity was decreased to 1/3 of the value for normal ($p < 0·001$) (Table I).

TABLE I

Digestive enzymes of pancreatic tissue of normal and thiamine deficient rats

	Group	Weight of pancreas	Amylase activity	Proteolytic activity	Lipase activity
Per g fresh weight of tissue	Normal	0·454±0·067*	5483±1108	62± 6	400±33
	Thiamine deficient	0·155±0·010	16983±3389	253±69	414±61
	p value	< 0·01	< 0·01	< 0·02	N.S.
Per mg tissue nitrogen	Normal	..	221± 39	2·5± 0·2	16·1± 1·3
	Thiamine deficient	..	499± 96	7·8± 2·1	13·3± 2·0
	p value	..	< 0·01	< 0·02	N.S.
Per whole pancreas	Normal	..	2490±503	28·5± 3·02	182±15·2
	Thiamine deficient	..	2632±525	39·3±10·82	64± 9·5
	p value	..	N.S.	N.S.	< 0·001

* Standard Error.   N.S.— Not Significant.

The results show that the amylase and proteolytic enzymes are not decreased in proportion to decrease in pancreatic weight or nitrogen content. The lipase on the other hand is decreased under these conditions and thus shows no change in specific activity. The study, therefore, does not indicate whether thiamine is directly involved in maintaining the enzymes of exocrine pancreas and further work is needed to explain the reason for the anorexia in thiamine deficiency.

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---

1. Wagner, A. F. and Folkers, K., *Vitamins and Coenzymes*, Interscience Publishers, London, 1964, p. 42.
2. Jollife, N., Goodhart, R., Gennis, J. and Cline, J. K., *Am. J. Med. Sci.*, 1939, **198**, 198.
3. Williams, R. D., Mason, H. L., Wilder, R. M. and Smith, R. F., *Arch. Intern. Med.*, 1940, **66**, 785.

4. Prasannan. K. G., Kondaiah, M. S., Kalyanasundaram, S. and Sundaresan, R., *Indian J. Biochem. Biophys.*, 1973, 10, 119.
5. Kunitz, M., *J. Gen. Physiol.*, 1947, 30, 291.
6. Somogyi, M., *J. Biol. Chem.*, 1952, 195, 19.
7. Nelson, N., *Ibid.*, 1944, 153, 375.
8. Clarke, J. M., *Experimental Biochemistry*, Freeman and Co., London, 1964, p. 55.
9. Varley, H., *Practical Clinical Biochemistry*, ELBS and W. Henneman Medical Books Ltd., London, 1969, p. 166.

BIOGENIC PROTODOLOMITE FROM THE LIMESTONES OF BOMBAY HIGH OIL FIELD

DURING the course of petrographic investigation of the limestones from the subsurface of Bombay High offshore oil field, small unit rhombohedra of the average size of 6 to 7 microns were noticed forming constituent particles of the micritic base of many micritic limestones. The grains are euhedral, water-clear, somewhat wine yellow in colour and devoid of any inclusions. The accompaying photomicrograph (Fig. 1) shows the morphology and mode of occurrence of the unit rhombohedra as a part of micritic base of the micritic limestones.

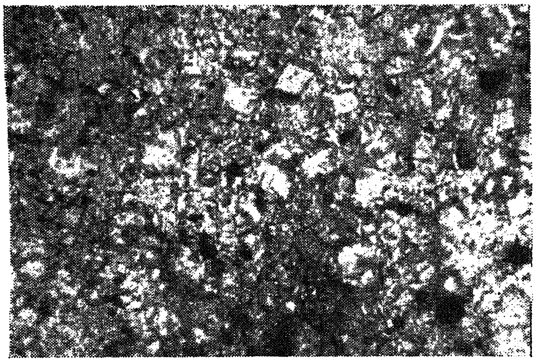

FIG. 1. Thin section of micritic limestone from a core sample of Bombay Offshore oil field showing protodolomite unit rhombohedra, × 400.

Figure 2 is an X-ray trace of a powder mount of a rock sample containing the unit rhombohedra. There is a prominent peak for calcite (3·04 Å *d*) and a peak in the range 2·93 Å *d* to 2·95 Å *d* spacings. On several X-ray traces of the same mount taken at different places and on mounts of other samples containing unit rhombohedra, the 2·93 Å peak was seen to shift its position down to 2·90 Å *d*. The $MgCO_3$ content in the mineral as read from Goldsmith *et al.* chart (1955)[2] is from 26 to 40 mol per cent in calcite. Dolomites reported from sedimentary rocks (Goldsmith and Graf, 1958) and from many modern hypersaline lakes (Bathurst, 1971)[1] contain about 5 mole per cent excess calcium than the requirement of a 1 : 1 stoichiometric dolomite (2·88 Å *d*) and therefore called by them as protodolomite. The nearest approach the mineral under study is the biogenic protodolomite reported by Schröeder *et al.* (1969)[5] from the tooth of a living echinoid. Due to lack of a better term, the mineral under report is also regarded as a protodolomite.

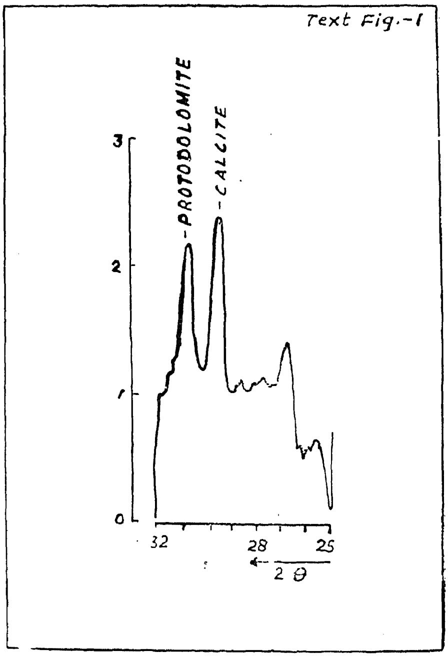

FIG. 2. X-ray chart at 20 2θ rotation per minute showing the peak position of calcite and protodolmite of the sample depicted in the micrograph.

Many invertibrate and plant marine organisms are composed of Mg calcites. Many algae and echinoderms contain Mg calcites in their skeletal architecture. Calcareous algae contain more than 20% Mg in their calcite and echinoderms about 40% (Millman, 1974)[4]. Besides, many skeletal parts of echinoderms contain different concentrations of Mg. Smaller spines contain larger amounts (Millman, 1974). In this connection it is interesting to note that in the thin sections of Bombay rocks, containing a large micrite base and abundant unit rhombohedra, there are very fine comminuted echinoderm skeletal fragments and many show grain diminution to minute unit rhombohedra. Millman (1974)[4] writes : "Many marine organisms precipitate magnesian calcite. During diagenesis the magnesium may be liberated and thus form magnesium rich interstitial waters which may be subsequently precipitated as dolomite." Such a process may be envisaged for Bombay High rocks also. For the first time, protodolomite of biogenic origin is reported from a geological formation.

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Kaulagarh Road,
Dehra Dun, *June* 21, 1976.

---

1. Bathrust, R, G. C., *Carbonate Sediments and Their Diagenesis,* Elsevier, 1971, p. 620.
2. Goldsmith, J. R., Graf, D. L., and Joensuu, O. I.; *Geochem. Cosmochemica Acta,* 1955, **7**, 212.
3. — and —, *Jour. Geol.,* 1958, **66**, 678.
4. Millman, J. D., *Marine Carbonates.* Springer-Verlag., 1974, p. 375.
5. Schröeder, J. H., Drownik, E. J. and Papkie, J. J., *Geol. Soc. Amer. Bull.,* 1969. **80**, 1613.

FEEDING ZONES AND INTERSPECIFIC ZONATION IN EARTHWORMS

THE feeding and subterranian activities of earthworms are not well understood, although their role in mobilization of nutritive substances in soil has been described in detail[1]. The present report describes the variations in the feeding and interspecific zonation of three species of earthworms available in and around Bangalore.

We have observed the activity of earthworms (100–200) in glass cages containing different layers of soils varying in organic matter (dry leaf litter) continuously for 3 weeks. Cages were filled with (*a*) Brown clay loam soil; (*b*) soils of varied concentration of organic matter, prepared from dry leaf; (*c*) organic matter prepared from different materials like hay, dung, mango, tamarind and guava leaves and wood ash in the proportion of 19 : 1 ; (*d*) wet sand at the bottom (2 kg) and soil containing dung matter (1·5 kg soil + 500 g dung).

Pheretima elongata burrowed deep in cages and aggregated as clumps invariably at 22″–28″ depth. *Megascolex mauritii* distributed throughout the length of the cage but showed a relatively greater aggregation at the depth of 4″–10″. *Pontoscolex corethrurus* was always seen in the subsurface within 5″ layer. This interspecific zonation was noticed only in cages with uniform composition of organic matter and it was lost when the cage was filled with different layers of soils varying in organic matter content.

All the three species showed preferential selection of a soil layer rich in humified matter in the cages containing different layers of soils for feeding. The mean rate of soil consumption varied with reference to the species, its size, nature of the soil and the organic matter content of the latter. These findings are in concomitant with those of Bhat *et al.*[2].

The feeding activity (in all the three species) was restricted to the dark phase of the day. During the light phase, the worms migrate to a layer poor in organic matter and settle there till nightfall. Thus they establish different zones for settlement and feeding.

Chemical analyses[3] of soils from these zones revealed a 2 to 5 fold increment in Ca^{++} and oxidizable C content in the settlement layer. Due to defaecation activity in this layer, the soil accumulates urea and non-protein nitrogen.

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1. Satchell, J. E., In *Soil Biology,* Ed. A. Burges and F. Raw, Academic Press, London and N.Y.. 1967, p. 259.
2. Bhat, J. V., Uma Bai and Subbannayya, K., *Curr. Sci.,* 1975, **44**, 654.
3. Jackson, M. L., *Soil Chemical Analysis,* Prentice-Hall of India Pvt. Ltd., New Delhi, 1967.

ISOENZYMIC PATTERN OF LACTATE DEHYDROGENASE IN VARIOUS TISSUES OF INDIAN PALM SQUIRREL, *FUNAMBULUS PENNANTI* (WROUGHTEN)

EARLIER studies have indicated that there are two primary types of lactate dehydrogenase (LDH ; EC 1.1.1.27) in most animals[1-3]. The two types are designated as H– and M–LDH which in different combinations make five isoenzymic forms. The LDH polypeptide subunits of vertebrates are encoded in at least two genetic loci, A and B[4]. On the other hand a third locus E functions in some fishes resulting in the formation of E_4 isoenzyme[5], whereas birds and mammals possess yet another locus C functioning in the primary spermatocytes[6-7]. The present report examines the isoenzymic pattern of LDH in various tissues of common Indian palm squirrel. *Funambulus pennanti* (Wroughten).

The squirrels were locally collected and kept for a week in the laboratory conditions. They were killed by cervical dislocation of the neck, and the organs spleen, muscle (gastrocnemius), heart and testes were immediately removed and frozen. A 10% (w/v) homogenate of each tissue was prepared in phosphate buffer (0·1 M, pH 7·4). The homogenate was then centrifuged at 14,000 × g for 30 mins. in Pr–6 International refrigerated centrifuge. The supernatant was collected and used as the source of enzyme. Protein content of this supernatant was determined[8] using Unicam Sp-500 spectrophotometer.

Polyacrylamide gel electrophoresis was carried out to study the pattern of LDH isoenzyme[9]. 7·5% gels were used and each was loaded with 100 μg of the enzyme source protein in 10% sucrose. The electrophoresis was then carried out at room temperature in tris-glycine buffer (0·1 M, pH 8·75) using 4 mA of the current per gel for 4 hours. After the run, gels were removed and LDH was specifically stained for localization[10]. The stained gels were photographed in transmitted light.

Figure 1 shows the pattern of LDH isoenzymes in testis, heart, skeletal muscle and spleen. In all these four tissues five isoenzymes are seen; M_4 near the origin and H_4 as the fastest moving band on the gels. The hybrids M_3H_1, M_2H_2, and M_1H_3 occupy the intermediate positions between the extremes. Two different patterns are visible, one shown by the testis and heart and the other by skeletal muscle and spleen.

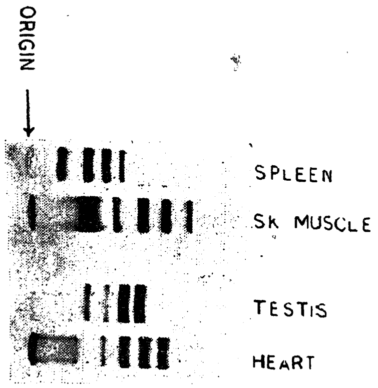

FIG. 1. Lactate dehydrogenase isoenzyme pattern in different tissues of *Funambulus pennanti*. Different bands are localized on the polyacrylamide gels according to the method described in the text.

In testis and heart, the preponderance of H_4 isoenzyme may be one of the adaptations of the enzyme to aerobic nature of the tissue. However, tissue specificity is seen in the difference in electrophoretic mobility of the enzyme in these two tissues. The other pattern of skeletal muscle and spleen shows M_4 to be the dominant isoenzyme. It is notable that the preponderance of M_4 is indicative of anaerobic tissues. Here also a difference in electrophoretic movement of the enzyme bands are apparent. Further, in spleen H_4 and M_4 are relatively lower in concentration as compared to skeletal muscle. The observed pattern of five isoenzymes bears resemblance to that of the rat tissues indicating the presence of two genetic loci in contrast to that of the lens and spermatocytes of fish and bird respectively.

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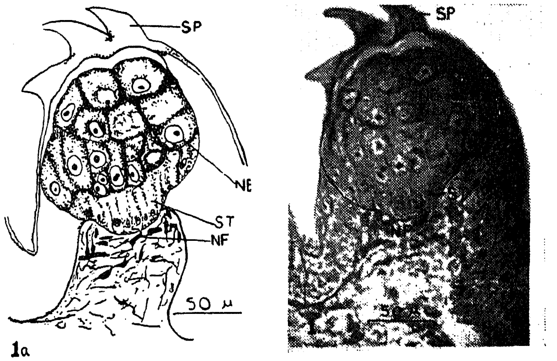
1. Appella, E. and Markert, C. L., *Biochem Biophys. Res. Comm.*, 1961, 6, 171.
2. Cahn, R. D., Kaplan, N. O., Levine, L. and Zwilling, E., *Science*, 1962, 136, 962.
3. Markert, C. L. and Ursprung, H., *Devel Biol.*, 1962, 5, 363.
4. —, *Science*, 1963, 140, 1329.
5. Whitt, G. S., *Arch. Biochem. Biophys.*, 1970, 138, 352.
6. Blanco, A. and Zinkham, W. M., *Scien* 1963, 139, 601.
7. Goldberg, E., *Ibid.*, 1963, 139, 602.
8. Sutherland, E. W., Cori, C. F., Haynes, R and Olsen, N. S., *J. Biol. Chem.*, 1949, 180, 825.
9. Davis, B. J., *Ann. N.Y. Acad. Sci.*, 1964, 121, 404.
10. Dewey, M. M. and Conklin, J. L., *Proc. Soc Exptl. Biol. Med.*, 1960, 105, 492.

A NEW SENSE ORGAN IN THE POSTERIOR LABIAL FOLD OF A TORRENTIAL FISH, *DISCOGNATHUS (GARRA) MODESTUS* (DAY)

THE presence of a unique sense organ, not hither reported in fishes, was observed in the epiderma region of the posterior labial fold of *Discognath* (*Garra*) *modestus* (Day) (Cypriniformes, Cyprinidae The sense organ was located in the blunt knob-lik projections of the epidermis. The sense organ spherical in shape and is about 48 μ–72 μ in dia meter. The two sides are demarcated by comm epidermal cells, whereas the front is guarded b curved cuticular spines. The basal portion supplied with nerve endings from the dermis situate just below it. The sense organ does not open the exterior by any pore. At least two types cells are distinct in it—the proximally situat large neuroepithelial cells and below these, t slender sustentacular cells (Figs. 1 and 1 a).

The neuroepithelial cells represent the main m of the sense organ forming a somewhat spheri body. These well-marked cells (length—14 μ–21 breadth—10 μ–17 μ) with prominent nuclei bigger than the other cells of the epidermis. sustentacular cells are slender and elongated. T are of varying lengths and breadths. Their nu are located in the basal portions of the cells.

Though the sense organ, under reference, resem a gustoreceptor by its location, nature of n supply and presence of neuroepithelial and sustentacular cells; yet it differs morphologically as well as histologically from the latter. Absence of opening to the exterior and structural differences of neuroepithelial and sustentacular cells of this sense organ from their counterparts in gustoreceptor speak of its difference from the gustoreceptor.



1a

The authors are of the opinion that the new sense organ may perhaps help the fish in detecting suitable substratum for firm anchoring at a definite spot against the swift currents of the torrential streams.

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MALE REPRODUCTIVE CYCLES OF *HEMIDACTYLUS FLAVIVIRIDIS* (RUPPELL) AND *UROMASTIX HARDWICKII* (GRAY)

The investigation reports the monthly variations in the weights and the histology of the testis and also the epididymal histology during the annual male reproductive cycles of two common house lizards, *Hemidactylus flaviviridis* (Ruppell) and the spiny tailed lizard, *Uromastix hardwickii* (Gray) inhabiting the semi-arid temperate climate. While *Hemidactylus* were collected locally, *Uromastix* were obtained from sandy tracts around Jaipur. In either case only freshly caputured healthy adult specimens were taken. Thus *Hemidactylus* weighing 5 to 9 g and measuring from snout to tail 10·3 to 14·8 cm, *Uromastix* weighing 68 to 101 g and measuring from snout to tail 21·5 to 32·5 cm were included in this study. Monthly collections of the two species were made round the year. At sacrifice, the testes and the epididymis were dissected and cleaned of all adherent tissues. Tissues were weighed and then fixed in Bouin's fluid, embedded in paraffin, sectioned at 6 μ and stained with Ehrlich's haematoxylin and 90% eosin.

Figure 1 presents the results obtained. For uniformity, histologic section of only that portion of the epididymis was used for the measurement of diameter of the tubules which was adjacent and attached midway to the length of the testes.

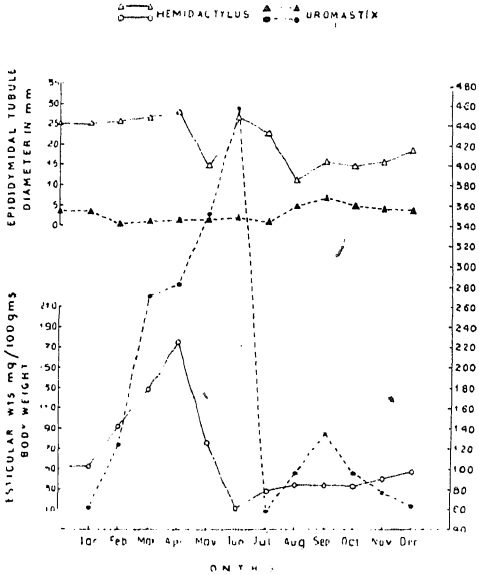

Fig. 1

In *Hemidactylus*, the testes are sexually quiescent and exhibit minimum weight in June and July. The testicular weight begins to increase from August and reaches a maximum in April. The peak in the testicular weight is followed by a decline which becomes statistically significant in June ($P < 0 \cdot 01$). A new spermatogenetic wave starts in July.

In *Uromastix*, the testes are sexually quiescent from October to January and exhibit minimum weight in January. The testicular weight begins to increase from July to September and starts decreasing from October till January. The increase in the testicular weight starts rapidly from February onwards reaching a maximum in June. The peak in the testicular weight is followed by a sudden statistically highly significant ($P < 0 \cdot 001$) decline in July. The decrease in testicular weight continues upto January when a new wave of spermatogenetic cycle starts.

In both the lizards the testicular histology compliments the seasonal changes in the weights of the testes. In *Hemidactylus*, in January, spermatogenic activity is initiated as a slow and gradual process with a sudden spurt during the month of April when the entire lumen becomes occupied mainly by spermatozoa. The interstitial cells appear as rounded or oval-shaped in appearance with distinct nuclei. By June–July, the animals become sexually inactive and the testis contains only spermatogonia although the interstitium has a large number of cellular elements. During the transitional period (August to December) the testicular histology exhibits the presence of the spermatogonial divisions and their subsequent transformations into the primary and the secondary spermatocytes. The spermatids appear in September and continue to exist during later months metamorphosing by December and January onwards into spermatozoa.

During the hiberanating months, the testes of *Uromastix* exhibit slow and progressive spermatogenesis. The interstitial cells are large with distinct nuclei. By March when the lizards emerge from hibernation, the animals are sexually active and remain so till June as is indicated by the active spermatogenesis in the testes and the availability of spermatozoa in the epididymal smears. The sudden and steep decline in the testicular activity starts in the last week of June and continues till the end of July. During this period of sexual inactivity, the interstitial cells are relatively few in number. The testis contains only spermatogonia and no other spermatogenetic elements. In specimens examined in the month of September, there is statistically significant increase in the testicular weight which also coincides with an increase in the diameter of the epididymical tubules.

In the present study a correlation between weight of testes, the diameter of the epididymal tubules and the availability of spermatozoa in the epididymis is noted during the annual reproductive cycle of both *Hemidactylus* and *Uromastix*. However, during June in *Hemidactylus* and September in *Uromastix* (Fig. 1) the mg testicular weight/100 g body weight appears anomalous since in these months the testes are dormant whereas the body weight is comparatively higher than that of March–April in *Hemidactylus* and of June in *Uromastix*. These observations coincide with the finding of Nice[5-6] and Saxena and Mathur[9] in the house sparrow.

The present observations on the male reproductive cycle of *Hemidactylus* and *Uromastix* agree in general with previous finding of Herlant[3], Mahendra[4] and Sanyal and Prasad[8] in *Hemidactylus*, Courrier[1] and Ramaswami and Jacob[7] in *Uromastix*. Between testicular activity and epididymidal tubules diameter, a similar relationship has also been reported in *Anolis carolinensis* (Fox[2]). Further he has noted that annual fluctuations in the diameter of ductus epididymis can be considered as a time indicator of the breeding season in *Anolis*. This is also found to be the case in *Hemidactylus* and *Uromastix* although the two lizards reveal markedly different patterns of male reproductive cycles. The variations in their reproductive patterns are probably due to the difference in their general behaviour and natural habits and habitant. Further work on these factors is in progress.

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---

1. Courrier, R., *Arch. Anat. Micr.*, 1928, **25**, 388.
2. Fox, W., *Copeia*, 1958, **1**, 22.
3. Herlant, M., *Arch. Biol.*, 1933, **44**, 347.
4. Mahendra, B. C., *Proc. Indian Acad. Sci.* (B), 1936, **4**, 250.
5. Nice, M. M., *Trans. Linn. Soc., N.Y.*, 1937, **4**,
6. —, *Condor*, 1946, **48**, 41.
7. Ramaswami, L. S. and Jacob, D., *Naturwissenschaften*, 1963, **50**, 453.
8. Sanyal, M. K. and Prasad, M. R. N., *Copeia*, 1967, p. 627.
9. Saxena, M. and Mathur, R. S., *Curr. Sci.*, 1976, **45**, 103.

MINERAL CONTENTS AND CHEMICAL DISSOLUTION OF THE POLYHEDRAL INCLUSION BODIES OF THE NUCLEOPOLYHEDROSIS VIRUS OF *AMSACTA ALBISTRIGA* WLK.

THE importance of chemical properties of inclusion bodied insect viruses has been reviewed by Smith[1]. The chemical analysis of polyhedral inclusion bodies (PIB) of nuclear polyhedrosis virus (NPV) of *Heliothis zea*, revealed the presence of several metals and nob-metals[2]. Though the occurrence of NPV in the groundnut red hairy caterpillar *Amsacta albistriga* has been reported and its cross infectivity tested in India, no information is available on the chemical properties of the PIB. In order to characterize the NPV of *A. albistriga*, studies were made on the mineral contents of PIBs as well as the effect of certain alkalies and organic solvents on their dissolution which are vital in understanding the mode of action of the virus in the insect.

The inclusion bodies were concentrated and purified from diseased final instar caterpillars of *A. albistriga* by differential cetrifugation. The PIBs (50 mg) were analysed for potassium and phosphorus contents by photometry, and calcium and magnesium by titrimetry[5]. Varian Tectran Atomic absorption spectrophotometer-120 was used to determine iron, copper, zinc and manganese in the triple acid extract. In the study on the effect of various alkalies and organic solvents on the PIB a drop of purified polyhedral suspension was placed on a clean slide, air dried, and dipped in various concentrations of alkalies and organic solvents. The dissolution of PIB was observed under a Meopta Phase contrast microscope for different periods after treatment, *viz.*, 5, 10, 15, 30 and 60 seconds, and 5, 10, 15, 30, 45 and 60 minutes.

The mineral analysis of PIBs of *A. albistriga* NPV revealed the presence of a higher content of calcium (1·6%), iron (0·6%), potassium (0·28%) and zinc (0·2%), and lower content of magnesium (0·05%), phosphorus (0·04%), copper (0·02%) and manganese (0·025%). The presence of magnesium and iron in the PIBs either by adsorption or as loosely bound salt linkage and possibility of major parts of phosphorus being associated with the crystallization of the polyhdral protein have been reported by Holoway and Bergold[6], [7]. Estes and Faust[8] have reported the presence of silicon in the PIBs which along with magnesium and iron may be responsible for the stability of the polyhdral. The fundamental unit of orthosilicate ion, SiO_4^{4-} in which the silicon atom is surrounded tetrahedrally by four oxygen atoms and balanced by the positive charges of cations, *viz.*, Fe^{++} and Mg^{++}, packed in the lattice in the interslices of the silicate ions. Thus the magneisum and ferric ions together with silicate ions constitute the silicaceous framework structure of the polyhedral matrix as postulated previously[8].

At a higher concentration of 0·2% of KOH and NaOH, the PIBs lost their refractile nature within 60 seconds and became black dense, dot-like granules, when viewed through the phase contrast microscope. No such effect was noticed in the lower concentration of 0·01, 0·05 and 0·1% of the alskalis. No dissolution of PIBs was found in 0·04, 0·4, 0·1 and 1 M concentrations of sodium carbonate, in 0·05, 0·1, 0·5 and 1 M concentration of sodium chloride, in 0·5, 1·0, 1·5 and 2% concentrations of sodium hypochlorite and in 5, 10, 15 and 20% formalin even an hour after treatment. The PIBs were also found insoluble in organic solvents like ether, chloroform, ethanol, methanol, propanol and butanol. The insoluble nature of PIBs in lower concentrations of alkalis but solubility in high concentrations of NaOH and KOH is in conformity with the earlier reports[9, 10]. Since there was no dissolution of PIBs even in 20% concentration of formalin, its use as an antiviral agent may be due to its penetrative effect suppressing the progressive infection[11], or denature of the viral DNA and/or modifying the inclusion body, thereby making occluded virions less accessible to susceptible larvae as suggested by Ignoffo and Garcia[12].

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---

1. Smith, K. M., *Insect Virology*, Academic Press, N.Y., 1967.
2. Shapiro, M. and Ignoflo, C. M., *J. Invertebr. Pathol.*, 1971, **17**, 449.
3. Jacob, A. and Subramaniam, T. R., *Curr. Sci.*, 1972, **41**, 436.
4. Rajamohan, N. and Jayaraj, S., *Ibid.*, 1975, **44**, 274.
5. Jackson, M. L., *Soil and Chemical Analysis*, Prentice-Hall, Englewood Cliffs, New Jersey, 1967.
6. Holoway, D. F. and Bergold, G. H., *Science*, 1953, **117**, 251.
7. — and —, *Ibid.*, 1955, **122**, 1266.
8. Estes, Z. E. and Faust, R. M., *J. Invertebr. Pathol.*, 1966, **8**, 145.
9. Pawar, V. M. and Ramakrishnan, N., *Indian J. Ent.*, 1971, **33**, 111.
10. Jacob, A. and Thomas, M. J., *Agri. Res. J. Kerala*, 1974, **12**, 82.
11. Vail, P. V., Henneberry, T. J., Kishaba, A. N. and Arakawa, K. Y., *J. Invertebr. Pathol.*, 1968, **10**, 84.
12. Ignoffo, C. M. and Garcia, G., *Ibid.*, 1968, **10**, 430.

ENHANCEMENT OF CHROMOSOMAL ABERRATIONS AND GROWTH INHIBITION BY MALEIC HYDRAZIDE WHEN DISSOLVED IN DIMETHYL SULPHOXIDE

Introduction

MALEIC hydrazide (MH) is known to induce localized chromosome breakages and mitotic suppression in *Vicia faba* root meristem[1,3,6,7]. It also resulted in inhibition of spindle formation and chromosome breakages during mitosis in the root tips of onion and barley[5].

MH is a pyrimidine component of RNA and a structural isomer of uracil. It is highly soluble in dimethyl-sulphoxide (DMSO). Therefore, the present experiment was undertaken to see the effects of MH alone (dissolved in water) and effect of MH and DMSO (when MH dissolved in DMSO) on barley root tips. Experiments were designed to find out the potentiating effect of DMSO on MH induced growth inhibition and chromosomal aberrations in barley root tips.

Materials and Methods

Seedlings with intact 2·5 ± 0·5 cm root tips were treated with 20 ppm of MH solution and 1 ppm of DMSO saparately for 4 hr (1 ppm of DMSO did not show any lethal or toxic effect on root tips). In another set of experiments root tips were treated with a mixture of equal volume of 20 ppm of MH and 1 ppm of DMSO for 4 hr. Each treatment was followed by recovery periods of 24, 48 and 72 hours. Handling of material, fixation, staining and detailed cytological techniques were the same as in earlier experiments with MH[5].

Observations and Comments

Chromosomes of barley do not possess well differentiated heterochromatin regions; therefore most of the breakages were concentrated at the centromeric region.

Predominant type of aberrations observed were spindle inhibition, chromosome breakages, anaphase bridges, stickiness and micronuclei found in 72 hr of recovery period after treatment. DMSO tried (1 ppm) induced very low frequency of anaphase bridges and stickiness in comparison to MH.

In DMSO, growth of the roots was increased from 2·5 to 4·2 cm upto 72 hr of recovery. In MH the increase in growth of roots was from 2·5 to 3·0 cm while in combined treatment it only increased 0·1 cm, *i.e.*, from 2·5 to 2·6 cm, thus showing that increase in growth was negligible in combined treatment. It has also the effect of loss of turgor and causing bends and curls in some roots. In higher recovery periods of combined treatment (72 hr) the root tips became hard, resembling swollen tumor like structure. Cytological analysis of such root tips showed highly fragmented chromosomes or nuclear blobs or the cells with dissipated nucleus. In extreme cases the entire nucleus may burst into small fragments of variable sizes.

Stickiness induced by MH may be due to depolymerization of nucleic acids[1] or it is an expression of latent sub-chromatid connections which did not open in the latter cell cycle. Higher frequency of bridges in cells which reached anaphase depicts that the chromosomes were experiencing a difficulty in separation at the telomere regions. Some of the cells at metaphase were showing groups of chromosomes scattered in the cells thus showing various stages of spindle inhibition. The basis of biological activity of combined treatment of MH and DMSO is not well understood.

Mode of action of MH and DMSO presumably involved interference with sulfhydryl groups which reduced the synthesis of those enzymes in which SH-groups were required. Therefore these chemicals effect -SH- and- SS- bonds in some way as a consequence of which the proteins necessary for the formation of spindle apparatus are also effected. MH also produces excess of reduced glutathione in actively growing root tip cells[4].

Thus the results summarized in this paper are considered strictly in relation to the information, these chemicals can give in causing more harm to the dividing cells. Because the positive advantage of this effect is in weed control, it is known that the MH effectively controls both monocot and dicot weeds and thus its weedicide property can be potentiated by dissolving it in DMSO solution. Barley seeds are exceptionally tolerant to the lower concentration of MH which are effective in causing damage to weeds.

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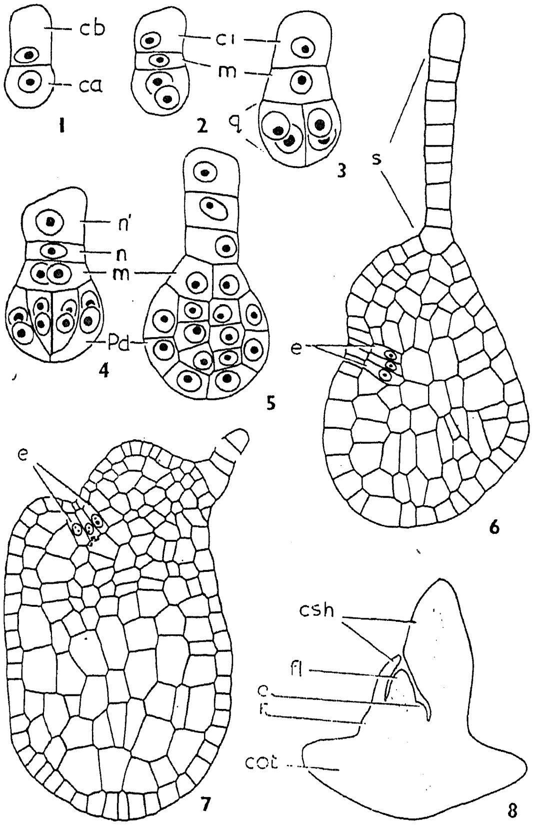
---

1. Darlington, C. D. and Mcleish, J., *Nature*, 1
167, 407.
2. Hughes, C. and Spragg, S. P., *Biochem. J.*, 1
70, 205.
3. Kihlman, B. A., *J. Biophys. Cytol.*, 1956, **2**,
4. —, *Action of Chemicals on Dividing* C
Prentice Hall, Inc., N.J. 1966.
5. Mann, S. K., S. Ved Brat and Sushil M
Acta Botanica Indica, 1974, **2**, 44.
6. Mcleish, J., *Heredity*, 1952, **6**, 125.
7. —, *Ibid.*, 1954, **8**, 385.

EMBRYO DEVELOPMENT IN *SCIRPUS MUCRONATUS* L.

A PERUSAL of literature reveals that the emb development in most of the cyperaceous t investigated so far, conforms to the Juncus varia of the Onagrad type[1]. On the contrary, an isola instance of the Asterad type has been reported Khanna[3] in *Scirpus mucronatus*. Her interpreta seems to be open to doubt[6,7]. Furthermore, data, on which Khanna's interpretation has b based, are apparently too fragmentary and ina quate to reach any such definite conclu Therefore it was felt desirable to reinvestigate embryo development in *S. mucronatus*, and o findings being different are presented here.

The details of embryo development in the ini phases up to the quadrant-stage have been fo to be essentially similar to those given by Khan However, a few relevant figures are included follow the cell-lineage (Figs. 1–3). The con tuent cells of the quadrant are divided by perici walls producing a group of 4 inner cells enclo by 4 protoderm cells and form an octant-emb (Fig. 4). Later, while the protoderm cells di only anticlinally, the inner cells undergo both tr verse and vertical divisions forming a glob proembryo (Figs. 5, 6). As the development ceeds, a depression appears on one side of spherical proembryo marking the beginning of formation of an annular cotyledonary sheath an the epicotylary locus situated at the base of depression (Figs. 6, 7). The remaining portio the embryo, distal to the notch, forms the cotyle Concomitant with these ontogenetic events the derivatives of the quadrant, the cell *m* u goes two vertical divisions at right angles to other and forms 4 circumaxial cells. The l by transverse divisions, give rise to the initia root cortex and root cap. The cells *n* and *n'* d transversely and form the suspensor of 4 to 10 (Fig. 6). As the embryo reaches maturity cotyledonary sheath enclosing the epicotyl an first leaf grows rapidly only on the side ad to the cotyledon. As a result, the opening c tubular cotyledonary sheath becomes highly oblique and eventually shifted to the basal position displacing the radicle to the lateral position. However, the cotyledon remains terminal (Fig. 8). In this respect, the present study confirms the earlier findings in other species of *Scirpus*[2,5,6,8].



FIGS. 1–8. Fig. 1. Two-celled proembryo. Fig. 2. Proembryonal tetrad. Figs. 3 and 4. Quadrant and octant-stages of proembyro. Fig. 5. Young globular proembryo. Figs. 6 and 7. Embryos showing the formation of depression. Fig. 8. Mature embryo.

(*ca*, terminal cell; *cb*, basal cell; *ci*, lower daughter cell of *cb*; *cot*, cotyledon; *csh*, cotyledonary sheath; *e*, epicotyl; *fl*, first leaf; *m*, middle cell; *n* and *n'*, upper and lower daughter cells of *ci*; *pd*, protoderm; *q*, quadrant; *r*, radicle; *s*, suspensor.)

As is stated earlier, Khanna[3] considered the development of embryo in *Scirpus mucronatus* to be conforming to the Asterad type. Her interpretation appears to have been based on the stages only up to the octant-embryo. In addition, she has not given any evidence on the role played by the basal cell in the formation of embryo, an important feature required to decide the embryonal type[1,4]. In contrast to Khanna's interpretation, the present observations show that the embryo development in *S. mucronatus* conforms to the Juncus variation of the Onagrad type[1].

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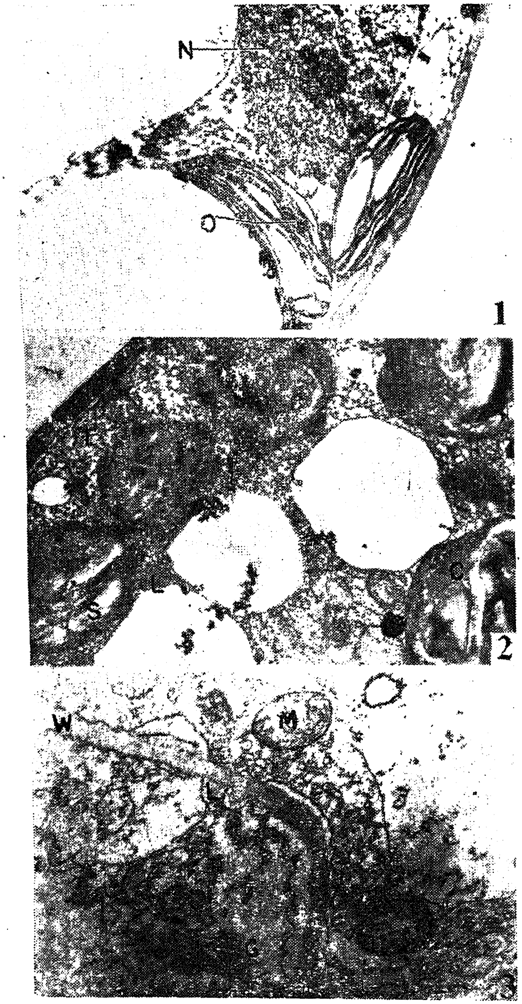
---

1. Johansen, D. A., *Plant Embryology*, Waltham, Mass., 1950, p. 116.
2. Juguet, M., *C.R. Acad. Sci. Paris*, 1966, 262, 1710.
3. Khanna, P., *Can. J. Bot.*, 1965, 43, 1539.
4. Maheshwari, P., *An Introduction to the Embryology of Angiosperms*, McGraw-Hill, New York, 1950, p. 271.
5. Nijalingappa, B. H. M., *Cytological and Embryological Studies in Cyperaceae*, Ph.D. Thesis, Bangalore University, 1970.
6. Shah, C. K. and Neelakandan, N., *Beitr. Biol. Pflanzen*, 1971, 47, 215.
7. —, In: *Advances in Plant Morphology*, Sarita Prakashan, Meerut, 1972, p. 102.
8. Van der Veken, P., *Bull. Jard. Bot. Etat. Belg.*, 1965, 35, 285.

EFFECT OF GIBBERELLIC ACID ON THE ULTRASTRUCTURAL MORPHOLOGY OF THE CELLS OF *LYGODIUM FLEXUOSUM* (L.) SW. GAMETOPHYTE

SOME interesting findings have come to light in the present study. No work has been done in this direction with respect to ferns. However, Ashour[1] *et al.*[1] (1974), studied the effect of GA on the ultrastructure of chloroplast and the content of chlorophyll in the cells of dwarf maize.

Spores of *L. flexuosum* were sown in 2 separate petridishes, each having approximately 500 spores in 1% Knop's sulution at 26° ± 2° C. Soon after the germination of spores (5 or 6 days) 2 mg/ml of GA solution was added to one of the petridishes and allowed to grow for 6 days. A few healthy gametophytes from each set were taken for ultrastructural studies for which the usual method for preparation of ultrathin sections was done. Silver or grey sections were mounted on uncoated copper grids and examined under Hitachi (Hu–IIE) electron microscope at 75 kv after double staining with 1% aqueous uranyl acetate and lead citrate. Ultrastructural studies of the cells of the control and the cells of GA treated gametophyte were undertaken. Our observations show that in the cells of GA treated gametophyte, endoplasmic reticulum increases and the cytoplasm gets densely filled up with it. Ribosomes, golgi bodies (Fig. 3), mitochondria and chloroplasts also increase in number as compared to that of control. The cristae in mitochondria swell up in the cells of GA treated gametophytes (Fig. 3). In cells of the control, chloroplasts are fewer; they are mostly spindle-shaped and have horizontally disposed lamellae with fewer starch grains and few osmiophilic bodie (Fig. 1). The ultrastructure of the chloroplast ge significantly altered in GA treated gametophyte cell as chloroplasts become more rounded with starch grains increasing in number and osmiophilic bodie getting reduced in size or totally disppearing (Fig. 2).



FIGS. 1–3. Fig. 1. Structure of different organelles in the cells of the control, × 14·800. Fig. 2. Structure of different organelles after treatment with GA. chloroplast (*c*) filled completely with ribosomes, lamellar structure is disturbed due to large starch grains. Starch grain (*s*) increase in size. Endoplasmic reticulum (E) completely filling cytoplasm. Lipid bodies (L) increased after treatment with GA, × 14·800. Fig. 3. Treated cell. Mitochondria (M) note the swelling of cristae of mitochondria at higher magnification. Golgi bodies (G) number gets increased after treatment with GA. Lipid (L) protrusion of wall and formation of lipid bodies. Wall of cell (*w*), × 26·000. N, Nucleus ; M, Mitochondria ; C, Chloroplast ; O, Osmiophilic bodies ; S, Starch ; W, Wall of cell ; G, Golgi bodies.

A few chloroplasts show disorientation of thei lamellae ; their grana thylakoids also swell up. Some chloroplasts get deformed to such an exten that even their fine lamellar structure cannot b made out.

Ribosomes within chloroplasts increase eno mously. Cell wall in controls appears perfectl stratified and uniformly thickened whereas in GA treated ones, it becomes more compact and get irregularly thickened with lipid deposition all alon its length (Fig. 3).

Increase in the number of mitochondri chloroplasts, ribosomes, endoplasmic reticulum gol bodies and lipid contents along the cell wall an within cytoplasma in the cells of GA treat gametophytes points towards increased activitie the cells. Probably GA helps in such biochemi activities which lead to increase in their numb consequently there is increased production carbohydrate, proteins and lipids. An increase the number of mitochondria probably indicat higher energy output by the GA treated cell Deformity of chloroplasts was also observed b Ashour *et al.* (1974). Increase in the number o osmiophilic bodies in the stroma of chloroplasts the cells of GA treated plants, observed by hi is not substantiated by our studies. On the con trary, we found that these bodies get reduced con siderably in number or they totally disappear within chloroplatst of the GA treated cell Distortion of lamellae may be caused by an increa in the number of starch grains which in their tu may cause certain amount of compression thereb distorting lammellar configuration of chloroplasts the GA treated cells.

The junior author is grateful to U.G.C. for t grant of a fellowship. The authors are thank to the Director, C.D.R.I., Lucknow, and to t electron microscopic section for help in electr microscopy.

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USHA KHER.

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1. Ashour, N. I., *Biochem. Physiol. Pflanz.*, 19 164 (4), 402.
2. Caulfield, J. B., *Biophys. Biochem. Cytol.*, 19 3, 827.

HOST RANGE OF *SAPROLEGNIA FERAX* (GRUITH) THURET ON CERTAIN FRESH WATER TELEOSTS

From previous reports *Saprolegnia ferax* has been found to be a virulent parasite of the fishes. Infection of *Saprolegnia ferax* has been reported from India on *Anabas testudinieus* (Bl.) by Bhargava[1].

During the course of investigations on fungi associated with fish diseases, *Labeo rohita* (Ham.), *Cirrhinus mrigala* (Ham.), *Ctenopharyngodon idella* (Val.), *Hypophthalmychthys molitrix* (Val.), and *Catla catla* (Ham.) were found to be infected with *Saprolegnia ferax* at Govt. Fish Farm, Gujartal, Jaunpur (U.P.), India, in February 1975. Studies were conducted in laboratory to establish the parasitic ability of this fungus and to investigate its host range.

Fungal mycelia were taken from living infected fishes and cultures were raised on boiled hemp-seed halves in sterile water. Unifungal bacteria free cultures were made on lines described by Raper[2], Tiffney[3] and Johnson[4]. Identification of the isolates was done with the help of monographs of Coker[5], Sparrow[6] and Seymour[7]. Fish species were identified using the keys of Srivastava[8] and Jhingran[9].

The parasitic ability of the fungus was tested on *Puntius sophore* (Ham.), *Colisa fasicatus* (Bl.), *Cirrhinus mrigala* (Ham.) and *Mystus tengara* (Ham.) by conducting artificial inoculation studies in laboratory on the lines described by Tiffney[10] and Scott[11].

The studies have established the parasitic ability of *Saprolegnia ferax*. Under laboratory conditions the hyphae appeared on the experimentally injured fishes in 12–18 hours. The death of *Colisa fasicatus* was caused in 27–36 hours of the start of the infection test, while in the case of *Puntius sophore* and *Cirrhinus mrigala*, the death was recorded on the third day. Infection studies of the fungus with *Mystus tengara*, gave negative results.

It has been observed that:

(i) Injury lowered the resistance of the fish to *Saprolegnia ferax*. (ii) The symptoms of the disease were nearly identical on various host species with respect to the behaviour of the infected fish and the appearance of the fungal tufts on the body of the host. Fungal infection of *Saprolegnia ferax* has been recorded on *Labeo rohita* (Ham.), *Cirrhinus mrigala* (Ham.), *Ctenopharyngodon idella* (Val.), *Hypophthalmychthys molitrix* (Val.) and *Catla catla* (Ham.). The pathogenicity of the fungus was established experimentally on *Colisa fasciatus* (Bl.), *Cirrhinus mrigala* (Ham.) and *Puntius sophore* (Ham.). Pathogenicity tests conducted with *Mystus tengara* (Ham.) gave negative results.

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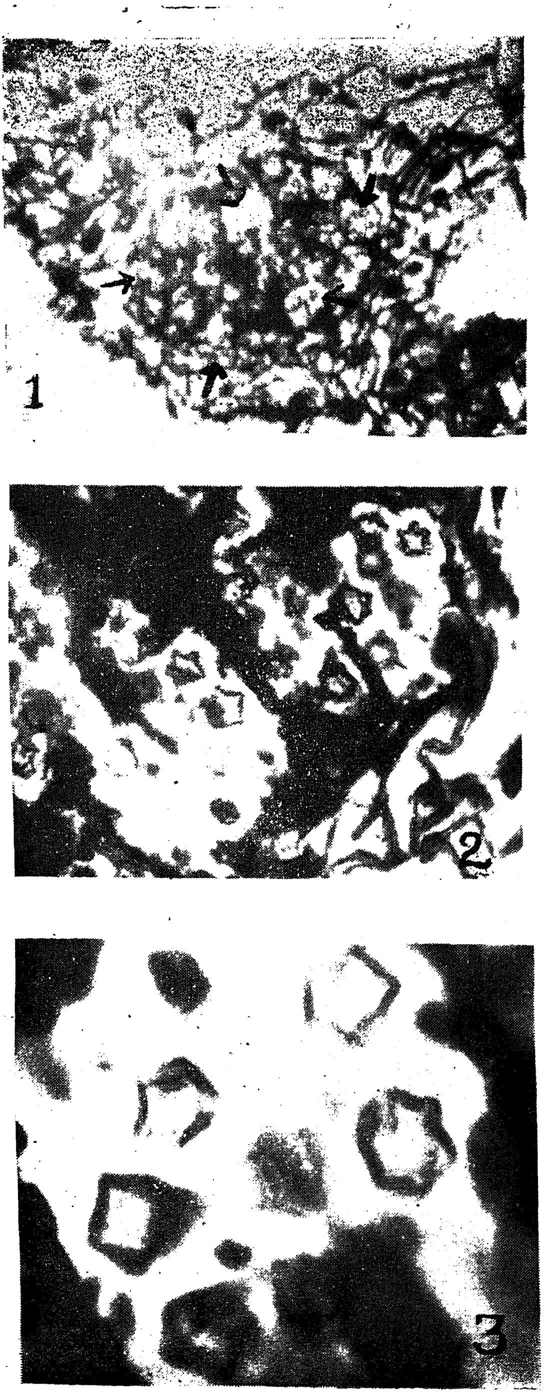
1. Bhargava, K. S., Swarup, K. and Singh, C. S., *Indian Biologists*, 1971, **3**, 65.
2. Raper, J. R., *Sci.*, 1937, **35** (2205), 342.
3. Tiffney, W. N., *Mycologia*, 1939, **36**, 413.
4. Johnson, T. W. Jr., *The Genus Achlya, Morphology and Taxonomy*, Univ. of Michigan Press, Ann. Arbor, Michigan, 1956.
5. Coker, W. C., *The Saprolegniaceae with Notes on Other Water Moulds*, Univ. of North Carolina Press, Chapel Hill. N.C., U.S.A., 1923.
6. Sparrow, F. K. Jr., *Aquatic Phycomycetes*, Univ. of Michigan Press, Ann. Arbor, Michigan, 1960.
7. Seymour, R., Howard, K. L. and Johnson, T. W. Jr., *Elisha Mitch. Sci. Soc.*, 1970, **86**, 63.
8. Srivastava, G. J., *Fishes of Eastern Uttar Pradesh*: Vishwa-Vidyalaya Prakashan, Bhairavanath, Varanasi (India), 1968.
9. Jhinran, V. G., *Fish and Fisheries of India*, Hindustan Publ. Corpn. of India, Delhi (India), 1975.
10. Tiffney, W. N., *J. Elisha Mitch. Sci. Soc.*, 1939, **55**, 134.
11. Scott, W. W. and O'Bier, *The Prog. Fish Cult.*, 1962, **24**, 3.

ON THE OCCURRENCE OF A NEW SYMBIONT IN *ANTHOCEROS*

The well investigated, widely distributed and essentially temperate genus *Anthoceros*, belonging to the class Anthocerotopsida of Bryophyta is represented in India by about 25 sps[1,2]. Some thalli of *Anthoceros* (species not determined) were collected from Kodaikanal, altitude about 2300 m. South India, during the annual Botanical excursion on 1st January 1972 and 1973 and this showed an interesting symbiont. Some materials were fixed in formalin acetic alcohol and some were collected in polythene bags for growing them in the green house. Gametophytes were observed in entirety and in following microtome selections customary methods. On examination, this species showed some interesting features, one of which was the presence of a new hitherto unrecorded symbiont in its mucilage cavities of the thalli. As is well known in this genus mucilage cavities which occur on the ventral side of the undifferentiated thalli are mostly inhabited by a cyanophycean alga, referred to the Nostocaceae and recorded as species of *Nostoc*. But in this species



FIGS. 1–3. Fig. 1. T.S. of thallus of *Anthoceros* showing mucilage cavities enclosing the colonies of symbiont *Tetraëdron*, "see arrow". Figs. 2 and 3. Mucilage cavities enlarged, showing the symbiont, pentangular bodies, *Tetraëdron* sp.

collected and referred to above, more than 40 t were examined and except in one or two case mucilage cavities did not contain *Nostoc* filam but unicellular forms referable to the Ch phyceae, order Protococcales, Family Oocyst subfamily Tetraëdrae. The alga agrees with characteristics of the genus *Tetraëdron*[3-6].

The genus *Anthoceros* has been represente about 25 sps. in India. A cursory exami showed that the species collected from Kodai may not be one of these known species, b it does not fully agree with any one of them. is also supplemented with the characteristic important feature that the mucilage cavitie comparatively, larger in size and the endo symbiont is not the usual *Nostoc*, but a green *Tetraëdron* with its conspicuous pentangular line.

In a few thalli the mucilage cavities in ad to these *Tetraëdron* a few *Nostoc* filaments ar noticed, while in others the *Nostoc* colonie completely absent and in their place, large n of *Tetraëdron* are found. Further work progress.

Sincere thanks are due to Prof. M. Nagara encouragement.

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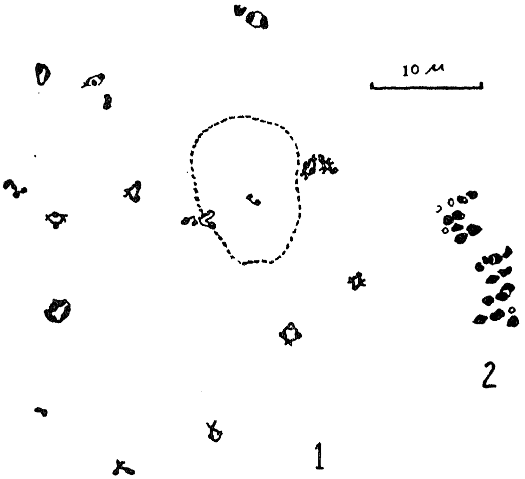
1. Chopra, R. S., "A Census of Indian Hep *J. Indian bot. Soc.*, 1943, 22, 237.
2. Fritsch, F. E., *Structure and Reproduct Algae,* Vol. 1. Cambridge, 1965.
3. Pande, S. K., *The Anthocerotales, Some A of Their Systematics and Morpl* Presidential address, Botany Section, Indian Science Congress, 1960.
4. Pascher, A., *Die Susswasserflora,* H Chlorophyceae : 2 Custav-Fischer-Jena,
5. Peirce, G. J., "*Anthoceros* and its Colonies," *Bot. Gaz.*, 1906, 42, 55.
6. Philipose, M. T., *Chlorococcales*, ICAR Delhi, 1967, 136.

SOME OBSERVATIONS ON THE CYTOLOG *CERAMIUM FIMBRIATUM*

THE species relationships among the red from a cytotaxonomic point of view, are impe understood. In an attempt to further our ledge on these lines, cytological studies were on some Ceramiales. The results obtained chromosome number and their behavio meiosis in *Ceramium fimbriatum* Setch & not reported in the literature[1,2], are presented

Small quantities of tetrasporangiate thalli, fixed in 1 : 1 glacial acetic aicd-rectified spirit solution, were smeared in aceto-carmine after pre-mordanting for two or three minutes in a weak solution of iron alum. The chromosome numbers and the types of their associations could be discerned without ambiguity in some of the nuclei at diakinesis and metaphase-I. The data presented below relate to such clearly analysable tetraspore mother cells (TMCs) in division.

At diakinesis, the chromosomes were associated as bivalents in a majority of nuclei. In others, variable numbers of univalents and polyvalents were observed, and in the latter case, the chromosome pairs were distinctly heteromorphic. Invariably more than one bivalent was associated with the nucleolus; occasionally terminal satellites could be observed in some of them. In a total of 25 nuclei analysed, 21 bivalents were met with in 16 TMCs while in the rest $2_{iv} + 17_{ii}$ or $1_{iv} + 19_{ii}$ or $1_{iii} + 19_{ii} + 1_i$ were observed, all of them giving the diploid chromosome number of $2n = 42$ (Fig. 1).



FIGS. 1–2 Fig. 1. Camera lucida drawing of a TMC at diakinesis showing $1_{iv} + 19_{ii}$. (Note heteromorphy of chromosome pairs in the quadrivalent). Fig. 2. Camera lucida drawing of a TMC at metaphase-I showing $19_{ii} + 4_i$. (Univalents unfilled).

At metaphase-I one or more polyvalents were observed in 10 cells while in the 19 other TMCs analysed, the chromosome associations ranged from $18_{ii} + 6_i$ to 21_{ii} per nucleus (Fig. 2). The $2n$ chromosome number of 42 and polyvlent frequency observed at metaphase-I correspond with those of diakinesis except for the higher number of univalents at metaphase-I. Secondary associations of non-homologous pairs could also be seen in metaphase-I nuclei.

Anaphase-I disjunction was normal with the chromosomes segregating individually rather than in clumps or plates reported for some red algae[1].

The high chhromosome number of $2n = 42$, occurrence of more than one nucleolar bivalent, formation of one or more polyvalents and heteromorphy of chromosomes involved in them, and the secondary associations of bivalents at metaphase-I suggest that this species is a polyploid of hybrid origin. The consistent diploid chromosome number of 42 and the normal disjunction at anaphase-I indicate that *C. fimbriatum* is a stabilized polyploid species on Visakhapatnam coast, and also that it is cytologically different from other Ceramiales studied so far[1,2].

We are thankful to the CSIR for the award of JRF to one of us (M. S.), and to Late Prof. T. Sreeramulu (Andhra University), for initiating us into these studies.

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1. Dixon, P. S., "The Rhodophyceae." In *The Chromosomes of the Algae*, Ed. M. B. Godward, Edward Arnold (P) Ltd., London, 1966, p. 168.
2. Magne, F., *Cah. Biol. Mar.*, 1964, 5, 461.

IN VITRO LIFE-CYCLE, REGENERATION AND APOSPORY IN *CERATOPTERIS PTERIDOIDES* (HOOK.) HIERON.

In vitro experiments have yielded useful information on many intricate problems of differentiation, development and life-cycle in aquatic ferns[1]. During our comparative *in vitro* studies on a few assorted aquatic ferns (both homo- and heterosporous), Ceratopteris pteridoides proved to be an excellent experimental material primarily owing to its shortest, generation-wise life-span.

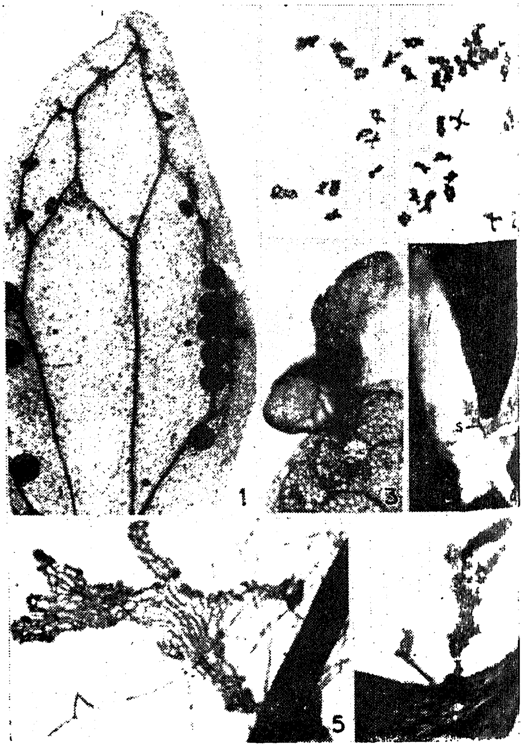
C. pteridoides is an aquatic annual recently reported to occur in China and south-east Asia[4]. Spores collected from Calcutta (India) were surface-sterilized in 1% aqueous calcium hypochlorite and sown on Knop's medium (full strength) prepared with glass-distilled water and solidified with 0·8% agar. The cultures were maintained under 12 hr photoperiod at 25 ± 2° C.

In 15–20-day old cultures, 3 distinct forms of gemetophytes were observed: (1) cordate, with two equally-developed, symmetrical lobes; (2) cordate, asymmetrical with two or more unequally-developed lobes and (3) spatulate with or without branches. In types (2) and (3), antheridia appeared first in 30–35 days and archegonia a week later rendering them hermaphrodite. The spatulate ones, on the other hand, remained antheridiate throughout their life-span. The fact that the 3 forms of gametophytes were observed persistently under uniform culture conditions make it certain that the differences observed in their adult phenotype and sex expression are indeed genetically controlled and in this respect they resemble with certain strains of *C. thalictroides*[5].

Fertilization was achieved in the presence of free water in the cultures and young sporophytes were observed 2 weeks after fertilization. During the vegetative phase which lasted for about 2 months, the earlier-formed leaves were deltoid with entire lamina and the sebsequent ones were 3-lobed. Thereafter, the fertile leaves, devoid of expanded lamina were differentiated; linear, undivided to begin with and 2–3 lobed later. As is known in other species of the genus, the sporangia developed on the underside of the fertile leaves protected by their reflexed margin. Interestingly, in some 7-month-old plants the factor(s) for the production of dimorphic fronds was/were lost because the sporangia were also formed on the expanded lamina of the vegetative leaves (Fig. 1). Meiosis (Fig. 2) and other stages of sporogenesis were perfectly normal resulting in 32 spores per sporangium. To the writers' knowledge, there is no report of *in vitro* completion of sporogenesis and subsequent stages of life-cycle in intact plants, a few earlier reports dealing with sporogenesis in excised leaves only[2,3].

Regeneration.—Vegetative leaves, both intact and excised, showed competence for bud formation on Knudson's medium; the degree as well as the sites of response were predictable. For example, while the buds produced on the juvenile leaves continued to produce vegetative leaves only, those on the adult leaves, which preceded the fertile ones on the parent plant, showed ability both for the differentiation of vegetative as well as fertile leaves. As to the site of response, groups of embryonic cells, situated near the leaf apices or below the sinus in the case of lobed ones, served as the bud initials (Figs. 3, 4). Thus in the absence of any exogenous growth factor in the medium, the bud development is related to the innate histological organization and physiological capacity of the plant.

Induction of apospory.—The object of this experiment was two-fold. First, to study the effect of starvation in favour of apospory; second, to assess the competence of apospory in intact *vs.* excised organs. Young sporophytes established on Knudson's medium containing 2% sucrose were transferred and maintained on the basal medium; no subculturing was done on the fresh medium. After 4 weeks the plants showed senescence followed by the differentiation of aposporous, diploid gametophytes from roots (Fig. 5), leaf lamina and stipe (Fig.
As regards the excised organs, while
roots of 1–3 hierarchial sequence failed
respond, the juvenile leaves of comparable seque
responded readily, as also reported earlier
C. thalictroides[7]. The aposporous, diploid gam



FIGS. 1–6. Fig. 1. Part of a morphologic vegetative leaf bearing normal sporangia in 7-month-old sporophcte, × 9. Fig. 2. Meio showing 40 bivalents at diakinesis, × 8. Figs. 3, 4, Regeneration of sporophytic buds fr the margin (3) and sinus of the leaf (4), S = veg tive, F = fertile leaf × 10, × 2. Figs. 5–6. A sporous, diploid gametophytes originating from root and stipe, × 19, × 10.

phytes generally tended to be intermediate in f
between types (2) and (3) of haploid gametoph
resulting from spores; the only notable differe
being the presence of tracheidal cells in some ca

Viewed as a whole, the results of the pre
study make it plain that both the sporophyte
gametophyte generation of this fern have the s
nutritional requirements for normal growth *in* v
Secondly, unlike in *Regnellidium diphyllum*[6], su
were not essential for normal growth.

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1. Allsopp, A., *Phytomorphology*, 1964, 14, 1.
2. Clutter, M. and Sussex, I., *Bot. Gaz.*, 1965, 126, 72.
3. Demaggio, A. E., *Amer. Jour. Bot.*, 1968, 55, 915.
4. Devol, C. E., *Amer. Fern. Jour.*, 1957, 47, 67.
5. Klekowski, E. J., Jr., *Bot. J. Linn. Soc.*, 1976, 63, 153.
6. Rao, A. N., *Flora*, 1966, 156, 378.
7. Singh, V. P., Roy, S. R., and John, B. M., *Commem. Vol.*, 1975.

INTERACTION BETWEEN MOSAIC VIRUS AND *FUSARIUM OXYSPORIUM*, f. sp. *PISI* (LINF) INFECTING PEA PLANTS (*PISUM SATIVUM* L.)

THE studies on the rhizosphere mycoflora of the plants infected with fungi and bacteria have been done by a number of workers, but similar studies on virus infected plants have received attention only during recent years. These include a number of virus-host combinations such as *Dolichos* sp., infected by dolichos enation mosaic virus[4], in coffee infected by decline disease[7], *Chenopodium album* infected by tobacco mosaic virus[5], croton chilli and tomato infected by croton yellow mosaic virus, chilli mosaic virus and tomato mosaic virus respectively[6], groundnut sB 11 infected by groundnut rosette virus[3]. The present paper however, deals with the effect of the virus on the population of *Fusarium* causing wilt in pea plants.

Sterilized and sieved soil was used for raising pea seedlings at the rate of five per pot. At the two leaf stage of the seedlings, the soil of each pot was infested with 10 ml spore suspension of *Fusarium oxysporium*, f. sp. *pisi* in sterilized distilled water and two days later the top leaf of each plant was inoculated with the standard virus extract. The inoculated plants were incubated in the glass house at 25° ± 2° C along with the control in which leaves were rubbed with distilled water. After 20 days of incubation the rhizosphere and rhizoplane were studied following the method of Laxmikumari[4], and the number of colonies of the fungus per gram oven dry soil were recorded in Table I.

It is evident from Table I that the virus enhances the population of the fungus both in rhizosphere and rhizoplane. R/r ratio of virus infected roots is almost the same as in healthy one. These ratios (R/r, D/H) indicate that fungal growth is proportional in both the cases. These results are in agreement with those reported by Buxton and Perry[1].

Possibly the altered physiological set up of the diseased and healthy plants may account for the differences in exudation and microenvironmental conditions of the root region which consequently affects the mycoflora differentially[2,3,4]. In most of the cases, an enhancement in the fungal population has been reported.

TABLE I

Number of Fusarium *colonies (per gram oven dry soil) in rhizosphere and rhizoplane of* Pisum sativum *L. var., bonneville infected with the virus*

Root	Rhizosphere (R)	Rhizoplane (r)	R/r
Diseased (D)	91·00	54·00	1·68
Healthy (H)	13·00	8·00	1·62
D/H	7:1	6·7:1	1·03:1

Therefore the variation in colony count may possibly be due to selective and differential assimilation of root exudates.

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1. Buxton, E. W. and Perry, D. A., *Trans. Brit. Mycol. Soc.*, 1959, 42, 378.
2. Chadha, K. C. and Raychaudhury, S. P., *Indian J. Agric. Sci.*, 1966, 36, 113.
3. Gangwane, L. V. and Deshpande, K. B., *Indian Phytopath.*, 1973, 26 (2), 325.
4. Laxmikumari, "Rhizosphere mycophlora and host-parasite relationship," *Doct. Thesis*, Univ. Madras, 1964.
5. Mishra, R. R., *Experientia*, 1969, 25, 657.
6. — and Kamal, *Indian Phytopath.*, 1970, 23 (i), 27.
7. Viswanath. N. R., Patil, R. B. and Rangaswami, G., *Curr. Sci.*, 1969, 38 (20), 497.

NEW RECORD OF ALTERNATE HOST PLANTS OF GROUNDNUT LEAF MINER *STOMOPTERYX SUBSECIVELLA* ZELLER (SYN.: *S. NERTERIA* MEYRICK) (LEPIDOPTERA: GELECHIIDAE)

GROUNDNUT leaf miner *Stomopteryx subsecivella* Zeller has been reported to attack redgram, *Cajanus cajan* Millsp.[1,2,4], soybean, *Glycine max* Merr.[1,4,5], green gram, *Phaseolus aureus* Roxb.[6] and a wild leguminous shrub, *Psoralea corylifolia* Linn.[1-4]

During the second week of November, 1974, *S. subsecivella* was observed for the first time mining the leaves of waste land weeds, *viz.*, *Indigofera hirsuta* Linn. (Leguminosae: Papilionaceae) and *Phaseolus calcaratus* Roxb. (Leguminosae: Papilionaceae) and the fodder plant lucerne, *Medicago sativa* Linn. (Leguminosae: Papilionaceae). A large number of leaf miner adults have migrated and oviposited on these plants soon after the harvest of groundnut during first week of November. A maximum of ten mines per leaf was observed on *I. hirsuta* whereas *P. calcaratus* and *M. sativa* had single mine on each leaf. Infestation on these plants continued upto December as furnished in Table I.

TABLE I

Incidence of groundnut leaf miner Stomopteryx subsecivella *Zell. in alternate host plants*

Sl. No.	Host plant	Percentage incidence			
		November 1973		December 1973	
		(1–15)	(16–30)	(1–15)	(16–31)
1.	*Indigofera hirsuta* Linn.	5·0	74·5	25·4	12·3
2.	*Phaseolus calcaratus* Roxb.	1·5	15·3	8·2	0·5
3.	*Medicago sativa* Linn.	Nil	5·0	2·6	Nil

It is seen from Table I that percentage of incidence in all the three host plants was high in the second fortnight of November.

S. subsecivella has not so far been reported on *I. hirsuta*, *P. calcaratus* and *M. sativa* and as such these hosts are new records.

The authors are grateful to the Director, Commonwealth Institute of Entomology, London, for identifying the insect specimens, to Dr. K. S. Krishna Shastry, Professor of Botany, University of Agricultural Sciences, Bangalore, for identifying the weeds and to the Indian Council of Agricultural Research, New Delhi, for the financial assistance.

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---

1. Ayyar, T. V. R., *Hand Book of Economic Entomology for South India*, Govt. Press, Madras, 1940, p. 211.
2. Fletcher, T. B., *Some South Indian Insects*, Madras, 1914, p. 457.
3. Lefroy, H. M., *Indian Insect Life*, Calcutta, 1909, p. 534.
4. Narayanan, E. S., "Insect pests of groundnut and methods of their control,"—In *Groundnut*, a monograph by C. R. Seshadri (ed.), 1961, p. 222.
5. Ordish, G. (Ed.), *PANS Manual* No. 2, Ministry of Overseas Dev., London, 1967, p. 43.
6. Prasad, S. K., Chawla, M. L., Kumar, S. and Saxena, H. P., *Indian J. Ent.*, 1971, 33 (1), 55.

POST-HARVEST FUNGAL DETERIORATION BANANA FRUITS

DURING studies on post-harvest diseases of fru a peculiar type of banana fruit rot was encount Apparently the fruits appear normal. How the symptoms become evident as soon as the are cut open. The disease is characterised by brown discoloration extending from top to b of the finger inside the pulp of the fruit (Fig

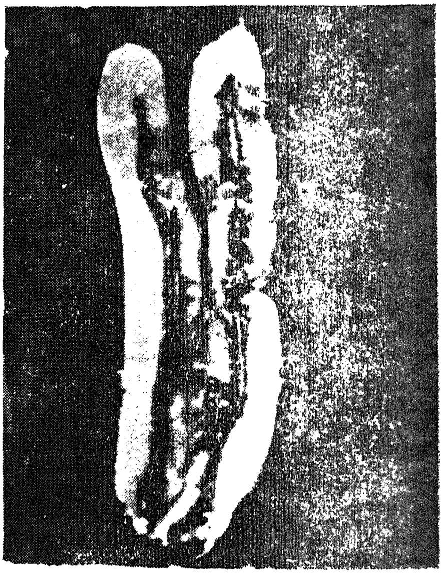

FIG. 1: Symptoms of banana fruit rot.

The infection in advanced stage has been ob to penetrate deep into the tissue which turn and pulpy. Isolations made from the di tissue yielded *Fusarium moniliforme*. The on inoculation of healthy fruits produced t symptoms.

Srivastava and Tandon[3] have described post-harvest diseases of banana from India. disease however, has not been reported from our country and is being described f first time. Similar type of malady has been rep from Israel by Temkin and Chorin[4].

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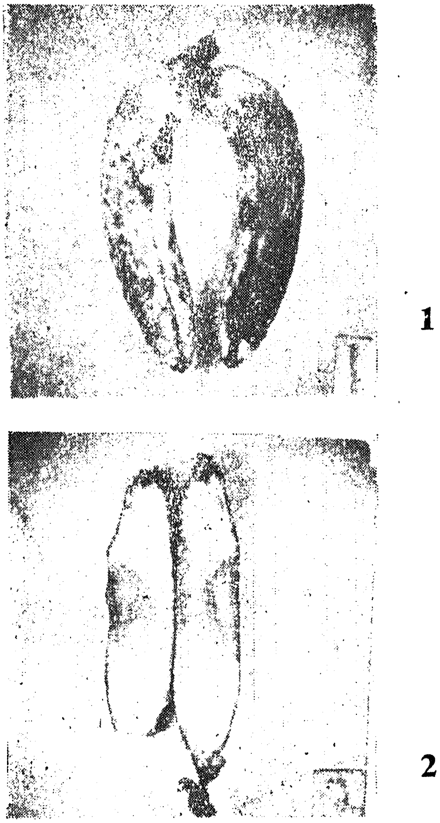
1. Dharam Vir, *Abst. Second Internationa Plant Pathology*, Indian Phytopath. S New Delhi, 1971, p. 99.
2. — and Gaur, A., *Curr. Sci.*, 1971, 40,
3. Srivastava, M. P. and Tandon, R. N., *Phytopath.*, 1971, 24, 115.
4. Temkin, Gorodeiski Naomi and C Mathilda, *Plant Disease Reptr*,. 1964, 48

A NEW FRUIT ROT DISEASE OF SINGAPURI VARIETY OF BANANA

DURING a local survey of the fruits in the markets and store-houses, an undescribed, severe soft rot of Singapuri variety of Banana was discovered and the causal organism was identified as *Fusarium solani*. The first symptom appeared on the skin of the fruit as scattered circular or elongated dark brown spots (Fig. I), ranging in size from small to 1·5 cm and as the disease progressed the entire fruit became soft and rotten (Fig. 2). The pathogenicity of the isolate was confirmed by various inoculation experiments of which the Koch's postulates were fully satisfied.



FIGS. 1–2. Fig. 1. Banana fruit showing natural infection. Fig. 2. Dissected fruit inoculated with *F. solani* showing internal rot.

The fungus identified as *Fusarium solani* (Martius) App. and Woll. has been confirmed by Dr. S. P. Raychaudhuri, Division of Mycology, IARI, New Delhi.

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ON THE OCCURRENCE OF *LERNAEOLOPHUS SULTANUS* (NORDMANN) ON *PRIACANTHUS HAMRUR* (FORSKAL)

Lernaeolophus sultanus is a cosmopolitan species of copepod, parasitic on marine fishes[1-6]. This parasite has been recorded from the Atlantic coast of U.S.A., Mediterranean, West coast of Africa and has thus a very wide distribution. The present communication is the first record of this parasite from the Indian waters barring a record from Ceylon.

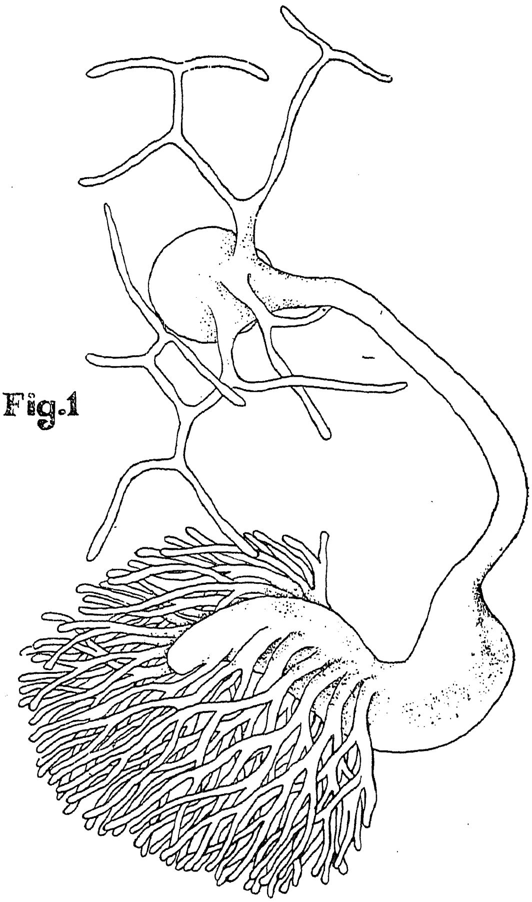

Fig.1

L. sultanus was found in the branchial cavity attached to the operculum of *Priacanthus hamrur* (Fig. 1). The only satisfactory illustration of the entire animal is that of Raibaut and Ktari[5], but the examination of the present material indicates that perhaps the specimens illustrated by them were not fully mature since the cephalic horns shown are shorter and less branched ; and the caudal processes fewer in number.

L. sultanus apparently exhibits variation in its external characters including shape of the body.

This is because as Kabata[3] observes "the definitive shape of the trunk of an adult female is an outcome of the adaptation to the particular type of host-parasite relationship, developed by individual genera. Although there are many and obvious exceptions to this rule, it can be maintained that the general trend is for the genera living exclusively on the outer surface of these hosts to retain a straight and elongate shape of the trunk. On the other hand it is probable that the genera with the sigmoid and flexed trunk have evolved in the more confined environment of the branchial chamber". How true this statement is can be seen by the shape of the body of the specimen illustrated here.

The cephalosoma of the parasite is directed towards the anterior end of the host and is completely buried in the opercular covering, and the attachment is made firmer by the dichotomously branched antlers present in the anterior side of the head. The branchial circulation of the gill filaments of *Priacanthus hamrur* can be occluded by the pressure exerted by the parasite. The presence of *L. sultanus* on the inner side of the opercular covering affects the movements of gills and reduce the efficiency of the outgoing respiratory currents. The trunk and the posterior processes, however, press against the gill filaments and lead to the eventual atrophy of many of the filaments.

The authors are grateful to Dr. N. K. Pillai, Professor, University of Kerala, for his kind assistance in the preparation of the illustration.

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1. Heller, C., *Zool. Teil.*, 1865, 2, 1.
2. Heegaard, P. E., *Rec. Austr. Mus.*, 1962, **25**, 149.
3. Kabata, Z., *Crustaceana Suppl. I.*, 1968, p. 103.
4. Kirtisinghe, P., *Parasitology*, 1935, **27**, 332.
5. Raibaut, A. and Ktari, M. H., *Bull. Inst. Oceanogr. Pech. Salammbo*, 1971, 2, 59.
6. Wilson, C. B., *Proc. U.S. nat. Mus.*, 1917, **53**, 1.

GENETICS OF BOAT-LEAF, NON-SCENTED AND OPAQUE KERNEL MUTATIONS INDUCED IN RICE

IN the course of an investigation on induction of mutations in different varieties of rice, three mutant characters showed segregation in M3 generation; based on segregation data, their pattern of inheritance was determined. One of the fine grain mutants, FG–14, induced in IR–8 (0·4% EMS treatment), segregated for boat-leaf plants in the proportion o 51 normal : 15 boat-leaf plants (3·4 : 1), an showed good fit to the expected ratio of 3 : 1 (X^2 = 0·185; P = 0·5–0·75). The boat-leaf mutan showed a thicker midrib which might be responsib for providing the needed mechanical strength an holding the lamina wings at an angle of 45°. Th boat-leaf phenotype along with associated compl of characters, *viz.*, lax panicle, long slender an awned grains, seems to be under the control of recessive gene.

In the scented cultivar HR–47, a dwarf mutan (*d*2) with scented grains was recovered in M generation, in 0·007 M *d*ES treatment. In M generation, however, it segregated for plants wi scented (109) and non-scented (32) grains (M ratio = 3·4 : 1; X^2 = 0·398; P = 0·50–0·70 giving a good fit to monogenic ratio. The segreg tion pattern suggests that loss of scent is governe by a recessive mutation. This result is in agreeme with those of earlier reports by Kadam and Patank (1938)[1] and Jodan (1944)[2].

In M2 generation, the seed of a promising sem dwarf mutant (Sd–4), induced by 0·4% EMS i the cultivar *Tellakattera* (with translucent grain showed clear translucent grains. In M3 generatio a total of 6882 seeds from ten randomly selec Sd–4 plants were screened, out of which 5171 ha translucent endosperm and the rest of 1711 ha opaque endosperm; the observed ratio was 3· translucent : 1 opaque (X^2 = 0·059; P = 0·7 0·90). Thus, opaqueness of the rice endosper seems to be governed by a single recessive ge Different workers, including Vander Stock (1910 Parnell *et al.* (1922)[4], Ramiah *et al.* (1931)[5] a Nagai (1959)[6] have reported that the opaq character of endosperm is determined by a recessi factor.

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1. Kadam, B. S. and Patankar, V. K., *Cu Bot.*, 1938, **4**, 496.
2. Jodan, N. E., *J. Am. Soc. Agron.*, 1944, 3 844.
3. Vander Stok, J. E., *Mededeelingen Uitgaa van het Department van Landbouw*, 19 12, 1.
4. Parnell, F. R., Rangaswamy Ayyengar, G. N Ramiah, K. and Ayyengar, C. R. S., *M Dept. Agric. India, Bot. Ser.*, 1922, 11, 1
5. Ramiah, K., Jobitharaj, S. and Mudaliar, S. T *Ibid.*, 1931, **18**, 229.
6. Nagai, I., *Japonica Rice; its Breeding a Culture*, Tokyo, Yokendo Ltd., 1959, p. 84

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and Printed at The Bangalore Press, Bangalore-560018.

SHAPE FACTOR ANALYSIS OF THE 690 keV BETA TRANSITION IN ^{111}Ag

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ABSTRACT

The earlier shape measurements on the 690 keV beta transition of ^{111}Ag are inconsistent with each other. So a thoroughly tested Siegbahn-Slatis coincidence beta ray spectrometer is used to study the shape of the above transition and the shape factor is fitted with a form $C(W) = k(1 + aW)$ where $a = (0\cdot0879 \pm 0\cdot0188)\ (m_0 c^2)^{-1}$. The results are discussed from a suitable model interpretation point of view.

INTRODUCTION

THE beta decay of ^{111}Ag is of particular interest both from the spectrum shape measurement and the nuclear model interpretation point of view. The presence of four betas with end point energies 1044, 793, 690 and 414 keV are well established[1,2,3]. Robinson *et al.*[2] employing a 4π anthracene spectrometer studied the shape of the 690 keV beta and reported a large deviation in the spectrum shape from linearity. Hamilton *et al.*[4] and later Seshagiri Rao *et al*[5] independently studied the beta-gamma angular correlation coefficient of the 690 keV beta in coincidence with the 342 keV gamma that followed it and reported the coefficients to be nearly independent of energy. Later Lehmann[6], employing a Siegbahn-Slatis coincidence beta-ray spectrometer reported the spectrum shape to be statistical in nature in complete contradiction with the earlier results due to Robinson *et al.* More recently Nagarajan *et al.*[7], employing an intermediate image spectrometer, confirmd the results of Lehmann. However, Nagarajan *et al.* employed a singles spectrometer and hence the shape analysis of the 690 keV beta, which was constructed after subtraction of two outer betas, involves large errors. Moreover the end point energy E_0 of the beta reported by Nagarajan *et al.* (*viz.* 695 keV) differs from the value reported by Lehmann, by as much as 10 keV.

Delabaye *et al.*[8] calculated matrix elements of this transition based on both extreme single particle model and experimental observables such as spectrum shape, beta gamma correlation and beta-circularly polarised gamma correlation. The matrix elements obtained from single particle model were unable to predict the shape deviation measured by Robinson *et al.* Seshagiri Rao analysed the matrix elements by relaxing the spectrum shape measurement of Robinson and Langer. He obtained the matrix elements of magnitude consistent with ξ-approximation where $\xi = \alpha Z/2R$. In fact the criterion for ξ-approximation $\xi >> W_0 - 1$ is well satisfied in this case ($\xi \sim 10$, $W_0 = 2\cdot3$). Thus it was felt that a remeasurement of the shape of the 690 keV transition would be interesting inasmuch as it clearst he discrepancies that exist in the earlier measurements and that it would also throw much light on the structure of the 343 keV level in ^{111}Cd.

EXPERIMENTAL DETAILS AND RESULTS

Carrier free ^{111}Ag isotope was obtained from BARC, Bombay. The decay scheme of ^{111}Ag is shown in Fig. 1. 2 mm diameter sources were prepared on thin mylar foils and the source thickness was found to be $\sim 150\ \mu g/cm^2$.

A Siegbahn-Slatis beta-ray spectrometer modified for beta-gamma coincidence studies was employed in the present investigation. The details of the modification and optimisation of the spectrometer for coincidence studies are described elsewhere[9,10].

The coincidence spectrum was recorded by scanning the beta spectrum in coincidence with the 342 keV gamma ray. The resolving time of the coincidence unit was set at 24 ns. The observed countrate was corrected for (i) chance rate, (ii) finite spectrometer resolution, (iii) back scattering and (iv) coincidence efficiency. Correction for beta-gamma angular correlation anisotropy was not applied as the reported anisotropy[5] is negligible. All the spectra were analysed with the Fermi functions of Bhalla and Rose[11] and screening corrections due to Buhring[12]. The method of analysis is described elsewhere[13]. The end point energy was determined from the behaviour of the shape factor curves, when E_0 the end point is varied around the true end point energy.

Figure 1 shows the Fermi-Kurie plot of the coincidence spectrum. The experimental shape factor was weighted least square fitted to a correction factor of the form $C(W) = k(1 + aW)$ and is shown by the solid line in Fig. 2. The values of 'a' for different runs are shown in Table I.

TABLE I

Run	Energy in keV	$a\ (m_0 c^2)^{-1}$
1	697±2	$-0\cdot0894 \pm 0\cdot0324$
2	696±2	$-0\cdot0836 \pm 0\cdot0312$
3	697±2	$-0\cdot0915 \pm 0\cdot0343$

DISCUSSION

^{111}Ag is a spherical odd-proton nucleus ($Z = 47$) which decays to ^{111}Cd. The neutron and proton configurations of ^{111}Ag are $50 + (2d_{5/2})^6\ (1g_{7/2})^6\ (3s_{1/2})^2$ and $28 + (2p_{3/2})^4\ (1f_{5/2})^6\ (2p_{1/2})^1\ (1g_{7/2})^8$ respectively. The initial and final configurations of the outermost beta with an end point energy of 1035 keV can be written as

$$[(\nu s_{1/2})_{J_1}{}^2 = 0 \quad \pi P_{1/2}]_{J' = 1/2}$$

and

$$[(\pi P_{1/2})_{J_2}{}^2 = 0 \quad \nu s_{1/2}]_{J = 1/2}$$

respectively.

Thus the daughter nucleus ^{111}Cd, where the odd neutron is in an $s_{1/2}$ orbital presents a good case for the applicability of the core excitation model due to de–Shalit[14].

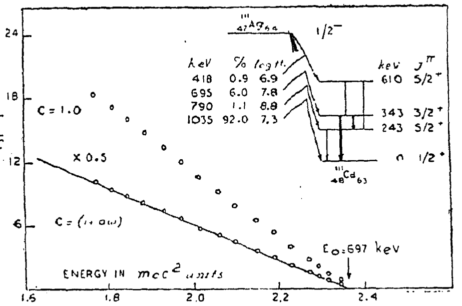

FIG. 1. The Fermi-Kurie plot of the 697 keV beta in the decay of ^{111}Ag measured in coincidence with the 343 keV gamma.

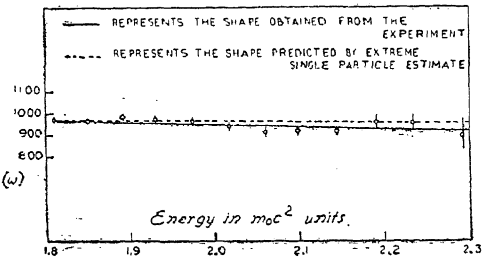

FIG. 2. Comparison of experimental and theoretical shapes of the 697 keV beta transition in ^{111}Ag.

The Nuclear matrix elements (NMEs) evaluated by both Delabaye *et al.* and Seshagiri Rao show that the normal matrix elements are reduced in size and are comparable to the B_{ij} matrix element. According to the ξ-approximation, the normal matrix elements dominate the transition and their magnitudes consequently should be larger than that of the B_{ij} matrix element. On the other hand if the modified B_{ij}-approximation is applicable, the reduction of the normal matrix elements must be so as to reduce them to magnitudes smaller than the B_{ij} matrix element. However, relative magnitude of the matrix elemen obtained by Delabaye *et al.* and Seshagiri Rao clearl indicates that none of these two extreme cases can be applied to the present beta transition. The cause o the reduction, however, cannot be inferred from th present experimental results inasmuch as the 'J- selection rule' is not violated and the 'K-selecti rule' cannot be invoked in view of the fact th ^{111}Cd is a spherical nucleus.

Delabaye *et al.* in their attempt to study the stru ture of the 340 keV state assumed a wave functi of the type:

$$| \mathrm{J},\ \mathrm{M} \rangle = \alpha \,|\, d_j\,;\ \ \mathrm{J} = j,\ \mathrm{M} \rangle + \beta \,|\, s_{1/2},\ \mathrm{J}_c = 2;\ \ \mathrm{J},\ \mathrm{M} \rangle,$$

in which, the first part corresponds to the single par cle excitation and the second part corresponds to core-excitation. The first part of the wave functi is assumed to be due to the excitation of the parti into the *d*-orbit. The later part of the wave functi corresponds to the retention of the particle in the orbit and exciting the core to the first phonon vib tional state. The relative amplitudes of these wa functions are denoted by α and β. Their analy however, showed that the values of matrix eleme were not sensitive to the magnitudes of α and β. pure de-Shalit type of core-excitation is, howe seldom encountered. Recently Rama Murty *et al* studied the states of ^{111}Cd using the intermed coupling model and described the $3/2^+$ state by following wave function:

$$| 3/2^+ \rangle = 0{\cdot}61 \quad | 0\ 3/2\ 3/2 \rangle - 0{\cdot}53\ | 2\ 1/2\ \ 3/2 \rangle + 0{\cdot}34\ | \ 2\ 3/2\ \ 3/2 \rangle - 0{\cdot}4\ | 2\ 5/2\ 3/2 \rangle + 0{\cdot}20 \,|\, 2\ 7/2\ 3/2 \rangle$$

and with this description all the electromagnetic p perties of this state were satisfactorily explained. the above wave function the contribution of the sin particle wave function $| 0\ 3/2\ \ 3/2 \rangle$ is 36%. present shape data are consistent with the si particle nature of the state involved. Noting the contribution of the $| 0\ 3/2\ \ 3/2 \rangle$ in the w function obtained by Rama Murty *et al.* is the m mum (36%), it may be concluded that the pre shape data are also consistent with the wave fur of Rama Murty *et al.* Alternatively, it may be pos to view the 343 keV state as arising from ang momentum coupling. The nucleus $^{111}_{48}Cd_{63}$ is of neutron type with 13 neutrons in excess of 50 (wi complete the first four major shells), having the orbi $d_{5/2}$, $g_{7/2}$, $s_{1/2}$, $d_{3/2}$, $d_{5/2}$ and $h_{11/2}$ for their occupati Several possible configurations are available in w a spin 3/2 could occur. A detailed study of th configurations may throw some light on the struc of the 343 keV state which may account for the re tion in the size of the normal matrix elements. present shape factor analysis is well in agreement the shape predicted by the matrix elements calculated by Delabaye *et al.* on the basis of extreme single particle model as shown in Fig. 2.

CONCLUSIONS

(1) The present experimental results regarding the shape of the 693 keV beta transition show a small deviation from the exact allowed shape reported by Lehmann, which is within the ξ-approximation. This might be due to the incorrect choice of the end point energy on the part of Lehmann.

(2) The present shape is consistent with the shape predicted by the matrix elements calculated from extreme single particle model.

1. Johansson, S., *Phys. Rev.*, 1950, **79**, 896.
2. Robinson, R. S. and Langer, L. M., *Ibid.*, 1958, **112**, 481.
3. Sastry, V. V. G., Lakshminarayana, V. and Jnanananda, S., *Nucl. Phys.*, 1964, **56**, 140.
4. Hamilton, J. H., Pettersson, B. G. and Hollander, J. M., *Arkiv Fysik*, 1960, **18**, 273.
5. Seshagiri Rao, V., *Ph.D. Thesis*, Andhra University, Waltair, 1967.
6. Lehmann, J., *Nucl. Phys.*, 1967, **A 98**, 273.
7. Nagarajan, T., Ravindranath, M. and Venkata Reddy, K., *Phys. Rev.*, 1971, **C 3**, 254.
8. Delabaye, M., Deutsch, J. P. and Lipnik, P., *Nucl. Phys.*, 1966, **80**, 385.
9. Ravindranath, M., Narasimha Rao, C. and Venkata Reddy, K., *Nucl. Phys. and Solid State Phys.* (*India*), 1972, **14 B**, 511.
10. Seshi Reddy, T., *Ph.D. Thesis*, Andhra University, Waltair, 1976.
11. Bhalla, C. P. and Rose, M. E., *ORNL Report*, 1962, No. 3207.
12. Buhring, W., *Nucl. Phys.*, 1965, **61**, 110.
13. Nagarajan, T. and Venkata Reddy, K., *Nucl. Instr. and Meth.*, 1970, **80**, 217.
14. de-Shalit, A. and Talmi, T., *Nuclear Shell Theory*, Academic Press, Inc., New York, 1963.
15. Rama Murty, V. V., Rama Rao, M. T. and Lakshminarayana, V., *Phys. Rev.*, 1970, **C1**, 1498.

SYNTHESIS OF SOME BENZOTHIAZOLYL HYDRAZONES

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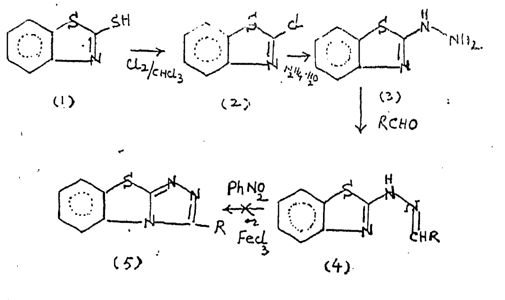
ALTHOUGH a large amount of work has been carried out on 1, 2, 4 triazoles, very little information is availabe on *s*-triazolo (3, 4-*b*) benzothiazoles possessing various functional groups. This gap may perhaps be due to non-availability of efficient methods for the synthesis of starting material. The method for the synthesis of this system involving the use of hydrazine has been discussed earlier by various workers[1,2]. Hydrazino benzothiazole is generally prepared from 2 mercapto[3] or 2-chloro benzothiazole by the action of hydrazine hydrate. The reaction was carried out with varying concentrations of hydrazine hydrate for different durations. There is no significant improvement in the yield. The reported chloro compound obtained from mercapto benzothiazole and sulfur monochloride was formed only in low yields. Heterocyclic thiols on oxidative chlorination are generally known to yield sulfonyl chlorides and this method finds a general use for the synthesis of the corresponding sulfonamides[4,5].

Hence the use of gaseous chlorine was investigated under mild condition. The conditions of reactions were so adjusted that the variation of both the time of reaction and the chloroform : water ratio in the suspending medium was possible. When gaseous chlorine was bubbled through mercapto benzothiazole suspended in chloroform and water it was noticed that the product after 50 to 60 min was indeed a 2-chloro benzothiazole. The chloro compound thus obtained was converted into the hydrazino compound in 72-75% yield. In this particular case reaction of chloro derivative with hydrazino, proceeded well to give good yields in contrast to some reports where the conversion of halo heterocycle to the corresponding hydrazine, proceeded with poor yields[6]; excessive heating with hydrazine[7] resulted in the decomposition of the mixed heterocycles, possessing sulfur and nitrogen. With excess of hydrazine for a period of 40 minutes, the yield is better than that reported by Veltman[8].



In the hope of obtaining biologically active compounds, hydrazino benzothiazole was condensed with various aldehydes. Cyclization of these hydrazones through nitrobenzene did not proceed and the oxidation with ferric chloride did not give satisfactory results. These were cyclized by bromine in acetic acid. Details of this will be published at a later date. Since heterocyclic compounds have featured prominently in biological activity, the compounds were screened for antifungal and plant growth activity. Benzothiazolyl hydrazones with heteryl substituents like pyridyl, furyl and thienyl were specifically selected in view of biological importance.

In the case of *T. vulgare*, the data presented in Table II indicate that the activity of benzothiazol-2-yl hydrazone of pyridine 3-aldehyde is equal to th of gibberellic acid and superior to 2, 4 dichlorophen acetic acid and other heteryl derivatives. In the ca of ***P. deveus*** benzothiazolyl-pyridine 3-aldehyde hyd zone is superior to all, as far as the percentage germination is concerned, though there is a slig decrease in the length of the radicle compared to No. It thus appears that among these three, the pyrid derivative is more active.

TABLE I

Benzothiazolyl-hydrazones

No.	R	Melting point °C	Yield %	Molecular formula	Analysis (found) C	H	N
1.	2-Hydroxy phenyl-	240	85	$C_{14}H_{11}N_3OS$	64·9 ;	4·09 ;	15·53%
2.	2, 4-Dihydroxy phenyl-	250	78	$C_{14}H_{11}N_3O_2S$	59·74 ;	3·71 ;	13·2%
3.	3-Hydroxy 4-methoxy phenyl-	182	78	$C_{15}H_{13}N_3O_2S$	59·92 ;	4·21 ;	14·11%
4.	*m*-Nitro phenyl-	236	86	$C_{14}H_{10}N_4O_2S$	56·89 ;	3·29 ;	18·34%
5.	2-Pyridyl-	220	61	$C_{13}H_{10}N_4S$	61·60 ;	3·96 ;	22·22%
6.	3-Pyridyl-	234	58	$C_{13}H_{10}N_4S$	61·8 ;	3·81 ;	22·89%
7.	4-Pyridyl-	224	80	$C_{13}H_{19}N_4S$	61·49 ;	3·56 ;	20·99%
8.	2-Thienyl-	210	77	$C_{12}H_9N_3S_2$	55·28 ;	3·58 ;	15·99%
9.	2-Furyl	180	74	$C_{12}H_9N_3OS$	58·88 ;	3·52 ;	17·51%

Solvent for crystallisation = Ethanol.

TABLE II

Effect of benzothiazol-2-yl (heteryl-aldehyde) hydrazone on germination of T. vulgare *and* P. deveus *seed*

No.	Name of the compound	*T. vulgare* Percentage germination	Length (cm)	*P. deveus* Percentage germination	Length (cm)
	Benzothiazolyl-				
1.	-Pyridine 3-aldehyde hydrazone	100	5	100	3
2.	-Furyl 2-aldehyde hydrazone	100	1·5-3	90	1·5–3·
3.	-Thiophene 2-aldehyde hydrazone	80	1·5–2·5	70	2–3
4.	Gibberellic acid (standard)	100	2–5	70	1–2·
5.	2, 4 dichlorophenoxy acetic acid (standard)	100	0·5–3	90	0·5–4·
6.	Control	90	2–3	70	1–2

Surprisingly one of the compounds was found to be very active as plant growth promoter. None these was found to be antifungal.

EXPERIMENTAL

2-Chloro benzothiazole (2)

2-Mercapto benzothiazole (1) (0·03 mole) was suspended in chloroform and water 2:1 mixture. The mixture was cooled to 5° C. Chlorine gas was passed for 1·5 hr. Chloroform layer was tapped off and the remaining liquid was distilled under reduced pressure (yield 2·8 g). B.P. 158–162° C/50 mm.

2-Hydrazino benzothiazole (3)

This is prepared by condensation of 2-chloro benzothiazole with hydrazine hydrate.

Benzothiazolyl hydrazones (4)

Equimolar proportions of 2-hydrazino benzothiazole and the required aldehyde in ethanol were condensed by heating on a water bath for 20–45 min. The reaction mass was poured into water. Crude hydrazone was filtered off and recrystallised from ethanol. The yields, melting points and analytical data, etc. are included in Table I.

The hydrazones were taken in nitrobenzene and refluxed for 2 hours. Then nitrobenzene is removed by steam distillation. The products were not the cyclised *s*-triazolo (3, 4-*b*) benzothiazole derivatives (5) but were found to be starting materials (4). Similarly ferric chloride oxidation also failed to give *s*-triazole (3, 4-*b*) benzothiazole derivatives.

Biological screening

After surface disinfection with a solution of sodium hypochlorite (2% available chlorine) for 10 min., the seeds of *T. vulgare* and *P. deveus* were soaked for five hours in the solution. Then seeds were sown on blotter in a petri dish. The blotters were sterilized at 15 psi for 20 min. before use. The blotters were moistened with 5 ppm solution of the compounds. Along with these compounds gibberellic acid and 2, 4-dichlorophenoxy acetic acid were also used as standards and sterile water was kept as control. The results are presented in Table II.

The author is indebted to Dr. V. R. Srinivasan, Professor of Chemistry, Osmania University, for continuous encouragement and helpful discussions and to Dr. K. S. Deshpande for biological testing. Author is also thankful to the U.G.C. for financial assistance.

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1. Soloveva, I. A. and Guseva, A. G., *Zhur Obschei. Khim.*, 1959, **29**, 2068.
2. Rangarao, V. and Srinivasan, V. R., *Experientia*, 1964, **20** (4), 200.
3. Boggust, W. A. and Cocker, W., *J. Chem Soc.*, 1949, p. 355.
4. Basu, N. K. and Rose, F. L., *Ibid*,, 1963, p. 5660.
5. Broadbent, W., Miller. G. W. and Rose, F. L., *Ibid.*, 1965, p. 3369.
6. Potts, K. T. and Huseby, R. M., *J. Org. Chem.*, 1966, **31**, 3538.
7. Rangarao, V., *The Chemistry of* 1, 3, 4 *Thiadiazole*, Thesis accepted by Osmana University, 1966.
8. Veltman, R. P., *Ukrain. Khim. Zhur.*, 1958, **24**, 351; *C.A.*, 1958, **52**, 52, 20673.

DETERMINATION OF STABILITY CONSTANTS OF 2-HYDROXY-1-NAPHTHALIDENE-*p*-N-DIMETHYLANILINE COMPLEXES WITH SOME BIVALENT METAL IONS

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ABSTRACT

Potentiometric studies have been carried on metal complexes of Cu^{+2}, Ni^{+2}, Co^{+2}, Zn^{+2}, Cd^{+2} and Mg^{+2} with 2-hydroxy-1-naphthalidene-*p*-N-dimethylaniline. The dissociation constants (pK_1 and pK_2) of the reagent and the formation constants of its metal complexes have been determined by Bjerrum's method at 30 ± 0·1° C and at ionic strength 0·1 M in 75:25% (v/v): dioxan-water medium. The order of stability is found to be Cu > Co > Ni > Zn > Cd > Mg.

LITERATURE survey indicates that no systematic study of the stabilities of 2-hydroxy-1-naphthalidene-*p*-N-dimethylaniline and its metal chelates with bivalent metal ions appears to have been carried out.

In the present communication the successive stability constants of the complexes of 2-hydroxy-1-naphthalidene-*p*-N-dimethylaniline with various bivalent metal ions have been determined potentiometrically following the Calvin-Bjerrum pH titration technique as adopted by Irving and Rossotti[1].

EXPERIMENTAL

The Corning Model 12, a precision research pH meter with a wide range glass electrode and a calomel reference electrode, was used for pH determination. The meter has an arrangement for normal and expanded scale. The smallest scale division on the expanded scale reads 0·005 pH unit.

2-Hydroxy-1-naphthalidene-*p*-N-dimethylaniline was prepared by dissolving 10 g of 2-hydroxy-1-naphthaldehyde (Fluka) in about 50 ml of absolute ethanol. To this solution was added equimolar quantity of p-N-dimethylaniline and the reaction mixture was refluxed on water-bath for about 20 to 30 minutes and then allowed to cool. On cooling, the compound separated out which was then repeatedly crystallised from ethanol to get an analytically pure compound with m.p. 168° C.

The dioxan used for the experimental work was purified by the method described by Vogel[2]. Distilled water was redistilled over alkaline potassium permanganate and made free from carbon dioxide by boiling. This water was used throughout the investigation.

The medium of titration was 75:25 dioxan-water (v/v) mixture. Perchloric acid and sodium perchlorate were added to maintain a constant ionic strength (0·1 M). The titrations were carried out in an inert atmosphere by bubbling oxygen-free nitrogen gas through the solutions. Metal ion solutions were prepared from A.R. grade metal salts. All these were standardised complexometrically by EDTA titrations[3]. All measurements were carried out at 30° ± 0·1° C.

The following solutions were titrated potentiometrically against standard carbonate free sodium hydroxide (1·092 M) solution keeping the total volume 40 ml.

(i) 5 ml of (0·16 M) $HClO_4$ + 5 ml of (0·64 M) $NaClO_4$ + 30 ml of dioxan. (ii) 5 ml of (0·16 M) $HClO_4$ + 5 ml of (0·64 M) $NaClO_4$ + requisite amount of reagent accurately weighed to give 0·01 M reagent concentration in the final solution + 30 ml of dioxan. (iii) 5 ml of (0·64 M) $NaClO_4$ + 5 ml of (0·024 M) metal salt solution in (0·16 M) $HClO_4$ + requisite amount of the reagent accurately weighed to give 0·01 M reagent concentration in the final solution + 30 ml of dioxan.

All titrations were performed in duplicate to test for reproducibility. The experimental method of Irving and Rossotti[1] was applied to find out the values of $\bar{n}$ and pL.

Results and Discussion

In the ligand, it is the chelated phenolic 'OH' group which takes part in the complex formation and the proton is replaced from it by metal ions during the formation of metal chelates. Since only one proton per ligand molecule is liberated during complexation, 'Y' the number of dissociable protons attached per ligand molecule is equal to one.

From the titration curves using the solutions (i) and (ii), $\bar{n}_A$ values at various 'B' values (pH meter readings) were calculated and the curve between 'B' and the corresponding $\bar{n}_A$ values was plotted (Fig. 1). The formation curve extends over a range $0 \cdot 51 < \bar{n}_A < 1 \cdot 7$ and is wavelike. This indicates the formation of the species HL and H_2^+L, *i.e.*, the protonated nitrogen and the phenolic hydrogen are completely dissociable in steps separable.

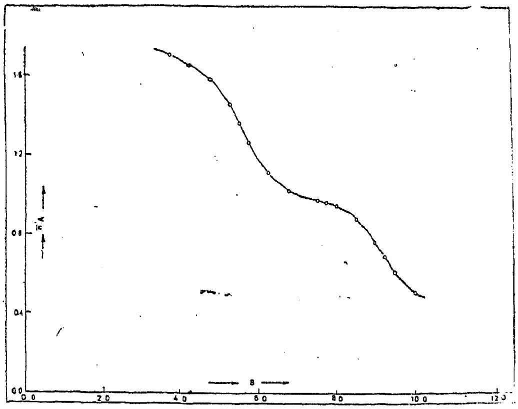

FIG. 1. 2-Hydroxy-1-naphthalidene-p-N-dimethylaniline formation curve.

Three methods known as half integral, graphical and least square[4] are suitably employed here to calculate the stability constants.

In the half integral method, the values of pL at $\bar{n} = 0 \cdot 5$ and $\bar{n} = 1 \cdot 5$ were taken as log K_1 and log K_2 respectively from the formation curves extending over a range of $0 < \bar{n} < 2$. In the cases where stepwise formation of the complexes is indicated by the flattening of the formation curves at integer values of $\bar{n}_A$ or $\bar{n}$, the different portions of 1:1 and 1:2 complexes were treated separately and the respective pK^H and log K values were determined by graphical method from the linear plots of

$$\log \frac{\bar{n}_A}{(1 - \bar{n}_A)} \text{ or } \log \frac{(2 - \bar{n}_A)}{(1 - \bar{n}_A)} \textit{ versus } \text{B (pH meter}$$

readings) and

$$\log \frac{\bar{n}}{(1 - \bar{n})} \text{ or } \log \frac{(2 - \bar{n})}{(\bar{n} - 1)} \textit{ versus } p\text{L as the case}$$

may be, by employing the relation

$$\bar{n}_A + (\bar{n}_A - 1) \, . \, pK_1^H \, . \, \frac{1}{\text{antilog B}} = 0$$

and

$$(\bar{n}_A - 1) - (2 - \bar{n}_A) \, . \, pK_2^H \, . \, \frac{1}{\text{antilog B}} = 0$$

and

$$\log K = \log \frac{\bar{n} - (i - 1)}{(i - \bar{n})} + p\text{L}.$$

In the cases where the formation curves are incomplete in the sense that they do not reach the value of $\bar{n} = 1 \cdot 5$ and in the cases in which the formation curves are not wavelike indicating that the formation of the second complex starts before the completion of the 1:1 complex, log K values are calculated by the least square method.

$$\bar{n} = \frac{K_1(L) + 2K_1K_2(L)^2 \ldots + NK_1K_2\ldots K_N(L)^N}{1 + K_1(L) + K_1K_2(L)^2 + \ldots K_1K_2\ldots K_N(L)^N},$$

This equation for 1 : 1 and 1 : 2 complexes can be written in the linear form as

$$\frac{\bar{n}}{(\bar{n}-1)\,L} = \frac{(2-\bar{n})}{(\bar{n}-1)} \cdot (L)\,K_1K_2 - K_1$$

knowing the quantities $\frac{\bar{n}}{(\bar{n}-1)(L)}$ and $\frac{(2-\bar{n})}{(\bar{n}-1)} \cdot (L)$ for each point on the formation curve the above equation is solved by the least square method to get the values of K_1 and K_2.

The value of pK_2^H only could be evaluated from the half integral point at $\bar{n}_A = 1 \cdot 5$ in the present case. The value of pK_1^H could not be found by half integral method. It was however determined by least square method.

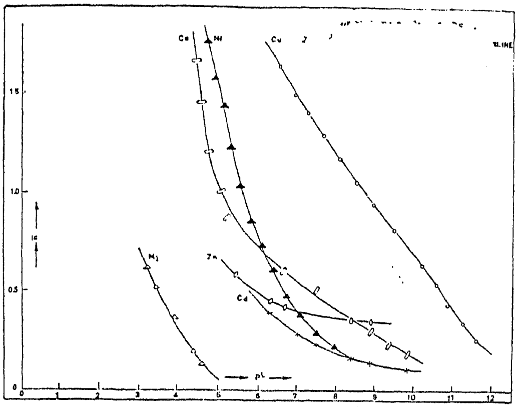

FIG. 2. Metal-Ligand system of 2-hydroxy-1-naphthalidene-p-N-dimethylaniline formation curves.

From the titration curves of the solutions (ii) and (iii), $\bar{n}$ and pL values were calculated. The $\bar{n}$ values were plotted against the corresponding pL values to get the formation curves of the metal complexion equilibria (Fig. 2). From these formation curves the values of stability constants log K_1 and log K_2 were determined which correspond to the pL values at $\bar{n} = 0 \cdot 5$ and $1 \cdot 5$.

Two plots one of log $\bar{n}/(1 - \bar{n})$ against pL (Fig. 3) and the other of log $(2 - \bar{n})/(\bar{n} - 1)$ against pL (Fig. 4) for different metal ion systems were also drawn. From these curves the values of log K_1 and log K_2 were evaluated. These values agree fairly well with those obtained from half integral method except in the case of cadmium where the log K_1 value had to be determined by graphical method on extrapolation of the curve. In addition the least square method was also applied to calculate log K_1 and log K_2 in the case of Ni^{+2} and Cu^{+2}. The most representative values are recorded in Table 1.

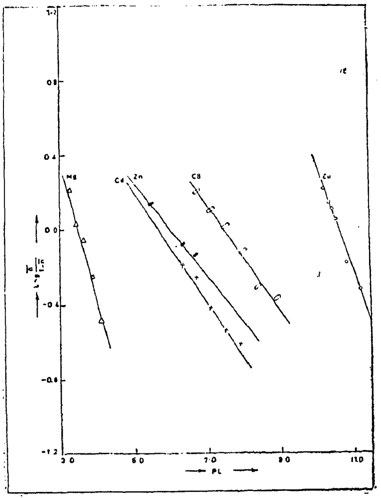

FIG. 3. 2-Hydroxy-1-naphthalidene-p-N-dimethyl-aniline.

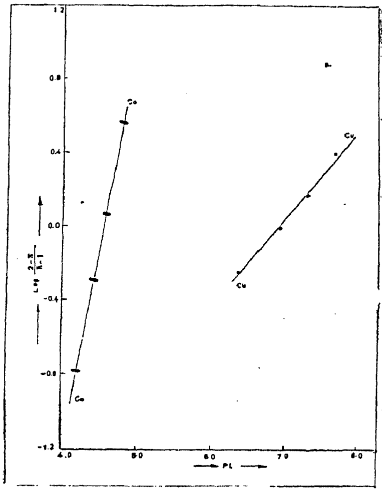

FIG. 4. 2-Hydroxy-1-naphthalidene-p-N-dimethyl-aniline.

TABLE I

*Stepwise stability constants of various complexes**

$t = 30°C$ $\mu = 0 \cdot 1$ M

Cations	H^+	Cu^{+2}	Ni^{+2}	Co^{+2}	Zn^{+2}	Cd^{+2}	Mg^{+2}
log K_1	9·59	10·65	6·76	7·35	5·95	5·73	3·50
log K_2	5·23	6·95	4·76	4·52	..	..	..

* For H^+, K_1 and K_2 correspond to the species LH and LH_2 respectively, while for the metal ions K_1 and K_2 correspond to the species ML and ML_2 respectively.

The order of stability of bivalent metal chelates was $Cu^{+2} > Co^{+2} > Ni^{+2} > Zn^{+2} > Cd^{+2} > Mg^{+2}$.

The order in the case of Co^{+2} complex is reversed with respect to Ni^{+2} complex, as compared to that observed by Maley and Mellor[5].

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The authors wish to record their sincere thanks to Dr. D. G. Vartak, B.A.R.C., Bombay, for his valuable suggestions, and to Dr. R. A. Kulkarni. Principal, Ramnarian Ruia College, for continuous encouragement in accomplishing this work.

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1. Irving, H. M., and Rossotti, H. S., *J. Chem. Soc.*, 1954. p. 2904.
2. Vogel, A. I., *A Text Book of Practical Organic Chemistry*, Longmans, III Edition, p. 177.
3. Schwarzenbach, G., *Complexometric Titrations*. Methuen and Co. Ltd., London, 1956, p. 60.
4. Irving, H. and Rossotti, H. S., *J. Chem. Soc.*, 1953, p. 3397.
5. Maley, L. E. and Mellor, D. P., *Nature*, 1947, **159**, 370.

FIRST INTERNATIONAL SYMPOSIUM ON AVIAN ENDOCRINOLOGY

THE First International Symposium on Avian Endocrinology, held at Calcutta, during January 10–15, 1977 was convened by Professor Asok Ghosh, Professor of Zoology of the University of Calcutta, and presided by Professor Donald S. Farner, the Chairman of the Department of Zoology, University of Washington, Seattle, U.S.A. The Symposium was inaugurated by Professor M. R. N. Prasad, Senior Professor of Zoology, University of Delhi. The inaugural session was presided by Dr. S. K. Mukherji, Vice-Chancellor, University of Calcutta. A number of renowned scientists from the U.S.A., U.S.S.R., Canada, U.K., Germany, Denmark, France, Holland, Hong Kong, Japan, Nepal, Bangladesh and different States of India participated in this symposium. The total number of delegates was around two hundred.

The Scientific Programme comprised of ten separate sessions, *viz.*, I. General Organisation of the Avian Endocrine system; II. Neuroendocrine Mechanism; III. Physiology of the pineal body; IV. Pancreatic islet and carbohydrate metabolism; V. Developmental endocrinology; VI. Mechanism of Hormone action; VII. Reproduction; VIII. Adrenal gland; IX. Thyroid and Parathyroid glands; X. Renal endocrinology; and XI. Valedictory session. Altogether sixty scientific papers were presented (twenty plenary lectures and contributed papers) during the 6-day symposium.

Both theoretical and applied aspects of avia endocrinology was discussed and the role of hormones on poultry and egg prduction was emphasized. A number of papers was presented on pes birds and birds which are agriculturally beneficial and there was a general opinion that a scientif inventory on this aspect would certainly help avia endocrinologists all over the world in th biological control of pest birds and an increase i the population of the beneficial birds.

LETTERS TO THE EDITOR

FORMATION AND REPROPORTIONATION CONSTANTS OF BINARY AND TERNARY COMPLEXES

STUDY of solution equilibria of mixed ligand complexes is of great importance, as this leads to the finding of the definite thermodynamic conditions for the compatibility of different ligands in the coordination sphere of the metal ion. In the present communication, we are reporting the values of reproportionation constant and the change in bond energies during the complexation of dipyridyl (dipy) phenanthroline (Phen) and thiotropolone (T.T.) with Ni^{2+}, Co^{2+} and Mn^{2+}.

Solutions of thiotroplone (T.T.)[1,2], 2-2 dipyridyl and O-phenanthroline were prepared in dioxan. The solutions of bivalent metal ions (Analar B.D.H.) were prepared in double distilled water and were standardised by usual methods. Sodium perchlorate was used to keep ionic strength constant ($\mu = 0 \cdot 1$ M). The following solutions (total vol. 20·0 ml) were titrated potentiometrically in 50% (v/v) dioxan medium, against 0·05 M tetramethylammonium hydroxide solution, using a Beckman pH meter (SS-2 model, in conjugation with a glass and calomel electrode assembly) at 30 ± 1° C.

a. (i) $HClO_4$ ($3 \cdot 0 \times 10^{-3}$ M); (ii) $HClO_4$ ($3 \cdot 0 \times 10^{-3}$ M) + T·T ($2 \cdot 5 \times 10^{-3}$ M); (iii) $HClO_4$ ($3 \cdot 0 \times 10^{-3}$ M) + T.T. ($2 \cdot 5 \times 10^{-3}$ M) + metal ion ($5 \cdot 0 \times 10^{-3}$ M).

b. (i) $HClO_4$ ($3 \cdot 0 \times 10^{-3}$ M); (ii) $HClO_4$ ($3 \cdot 0 \times 10^{-3}$ M) + T.T. ($5 \cdot 0 \times 10^{-4}$ M); (iii) $HClO_4$ ($3 \cdot 0 \times 10^{-3}$ M) + dipy or phen ($5 \cdot 0 \times 10^{-4}$ M) + metal ion ($5 \cdot 0 \times 10^{-4}$ M); (iv) $HClO_4$ ($3 \cdot 0 \times 10^{-3}$ M) + dipy or phen ($5 \cdot 0 \times 10^{-4}$ M) + T.T. ($5 \cdot 0 \times 10^{-4}$ M) + metal ion ($5 \cdot 0 \times 10^{-4}$ M).

Using Irving and Rossotti[3] method, the value of $\bar{n}$ and pL of the different binary and ternary complexes have been calculated from titration sets (*a*) and (*b*) respectively. The corresponding values of stability constants, $\beta_{0,1}$ and $\beta_{0,2}$ for different binary complexes and $\beta_{1,1}$ for ternary complexes were obtained using weighted least squares method of Sullivan *et al.*,[4] with the help of IBM 360 computer. The S_{min} values (χ^2) have also been calculated[5].

Reproportionation Constant[6]. The formation constant of the mixed ligand complex is directly proportional to the geometrical mean of the formation constants of the parent complexes, and the proportionality factor is termed as Reproportionation constant (K_d) *i.e.*,

$$\beta_{j,i} = K_d \cdot \beta_{m,o}^{j/m} \cdot \beta_{o,m}^{i/m} \qquad (i + j = m)$$

When the ligands L and A are not compatible the mixed ligand complex formed is less stable than the parent complexes and the value of reproportionation constant is smaller than unity, whereas in compatibility of the ligands, it is greater than one. The results of present investigations are given in Tables I and II.

TABLE I

Stepwise stability constants of various T.T. complexes at 30 ± 1° C

Metal ion	$\log k_1$	$\log k_2$	$\log \beta_{0,2}$
H^+	7·20	..	..
Ni^{2+}	6·75	5·81	12·56
Co^{2+}	6·20	5·70	11·90
Mn^{2+}	5·80	4·85	10·65

$\log k_1$ and $\log k_2$ values for Cd^{2+} could not be calculated as the binary complexes of T.T. are insoluble in 50% dioxan.

TABLE II

Formation and reproportionation constants of mixed ligand complexes with S_{min} values at 30 ± 1° C

L	M^{2+}	$\log \beta_{1,1}$	S_{min}	K_d
Bipyridyl	Ni^{2+}	6·73	2·1	0·52
	Co^{2+}	6·16	0·10	0·52
	Mn^{2+}	5·70	1·79	0·61
	Cd^{2+}	5·85	7·00	..
Phenanthroline	Ni^{2+}	6·72	0·79	0·45
	Co^{2+}	5·92	0·39	0·45
	Mn^{2+}	5·29	0·25	0·56
	Cd^{2+}	5·79	1·5	..

TABLE III

Change in bond strengths of various mixed complexes (cal/mole) at 30 ± 1° C

L	M^{2+}	M–L (parent)	M–L (mixed)	Change in bond strength	M–T.T. (parent)	M–T.T. (mixed)	Change in bond strength
Bipyridyl	Ni^{2+}	4580	2342	−2238	4337	2308	−2029
	Co^{2+}	3876	2010	−1866	4129	2245	−1884
	Mn^{2+}	2798	1403	−1395	3678	2535	−1143
Phenanthroline	Ni^{2+}	6078	2960	−3118	4337	1682	−2655
	Co^{2+}	4800	2580	−2220	4129	1510	−2619
	Mn^{4+}	2832	1295	−1537	3678	2297	−1381

***Bond Strengths*[6]:** The strengths of the individual M–A and M–L bonds in the complex MA_jL_i have been calculated from the formation constants of the mixed complexes and the parent MA_j and ML_i complexes using the following relations:

$$F_L' = \frac{RT}{4_j}\ln\beta_{j,i}\frac{\beta_{j,0}}{\beta_{0,i}}\ ;\ F_A' = \frac{RT}{4_i}\ln\beta_{j,i}\frac{\beta_{0,i}}{\beta_{j,0}}$$

while the bond strengths of the parent complexes MA_m (FA) and ML_m (F_L) were calculated from their formation constants using equations:

$$F_L = \frac{RT}{2_m}\ln\beta_{m,0}\ ;\ F_A = \frac{RT}{2_m}\ln\beta_{0,m}$$

The values, thus obtained, are reoorded in Table III.

In the present investigations, it is found that the value of reproportionation constant for mixed complexes is less than one and the bond strengths of all the metal to ligand bonds decrease, showing that the ligands are incompatible and the mixed complex is less stable than either of the parent complexes.

In the case of Ni^{2+}, the formation constants of the mixed complexes are not significantly lowered, as observed in other cases. This may be attributed to the fact that Ni–N bonds of Ni-Dipy complexes have some amount of M → L $d\pi$–$p\pi$ interaction along with the L→M bond[7].

The mixed complexes of phenanthroline show similar trends as dipyridyl complexes. But because of its greater size, their formation constants are slightly lower than the formation constants of dipyridyl complexes.

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1. Nozoe, T., Sato, M. and Matsui, M., *Proc. Japa Acad.*, 1953, **29**, 22.
2. —, — and —, *Ibid.*, Tohoku Univ., Ser. I, 195 **37**, 211.
3. Irving, H. M. and Rossotti, H. S., *J. Chem. Soc.*, 1954, p. 2904.
4. Sullivan, J. C., Rydberg, J. and Miller, W. F. *Acta Chem. Scand.*, 1959, **13**, 2023.
5. Rydberg, J. and Sullivan, J. C., *Ibid.*, 1959, 1 2059.
6. Fridman, Ya. D., *Proc. Symp. Coord. Chem.*, Hungary, 1970, p. 79.
7. Heureaux, A. L'G and Martell, A. E., *J. Ino Nuc. Chem.*, 1966, **28**, 481.

CIS-HYDROXYLATION OF OLEFINIC COMPOUNDS WITH SILVER SUCCINATE AND IODINE

SILVER salts of carboxylic acids and iodine have been employed for the preparation of *vic*-glycol from olefinic compounds. Prevost[1] showed that the complex formed from silver benzoate (2 moles) and iodine (1 mole), reacted with an olefin to give the corresponding *vic*-glycol dibenzoate. This reaction has been made use of by Witcoff and Miller[2] and Raman[3] for the preparation of *vic*-dihydroxy acids from typical monoethenoid acids, the products being the corresponding *threo*-dihydroxy compounds when dry benzene is used as the medium for the reaction.

It has now been found that the use of silver succinate and iodine in molecular proportions in dry benzene medium is a very efficient method of preparing *vic*-dihydroxy acids from olefinic acids. Oxidation of octadec-9-enoic acid and of docos-13-enoic acid thus has given 9, 10-dihydroxy stear

TABLE I

Monoethenoid acid oxidised	Dihydroxy acid obtained	Yield %	Melting point Found °C	Melting point Reported °C	Product by using silver benzoate and iodine with yield %
cis-octadec-9-enoic acid	*erythro*-	72	132	132	*threo*-, 60
trans- ,, ,, ,,	*threo*-	65	94	95	*erythro*-, 65
cis-docos-13-enoic acid	*erythro*-	78	130–31	131	*threo*-, 56
trans- ,, ,, ,,	*threo*-	82	100	100	*erythro*-, 72

acid and 13, 14-dihydroxybehenic acid respectively in very good yields, the *cis*-monoethenoid acid giving the *erythro*-dihydroxy acid and the corresponding *trans*-isomeride giving the *threo*-isomer. Thus hydroxylation using silver succinate and iodine in dry benzene involves *cis*-addition of hydroxyl groups, whereas, hydroxylation using silver benzoate and iodine in dry benzene involves *trans*-addition of hydroxyl groups. The results obtained with the different monoethenoid acids are tabulated in Table I and compared with the results obtained by hydroxylation of the acids using silver benzoate and iodine in dry benzene medium.

General Oxidation Procedure.—Dry silver succinate (0·011 mole) was mixed with 70 ml of sodium-dried benzene and kept under gentle reflux in a water bath with calcium chloride guard tube. Dry, powdered iodine (0·01 mole) was added in three lots and the refluxing continued until the colour of iodine disappeared (*ca* 60 min.). The dry, unsaturated acid (0·01 mole) dissolved in 5 ml of dry benzene was then added and the mixture was gently refluxed for 6 hrs. After filtration, the benzene was removed from the filtrate and the residue refluxed with alcoholic potash (50 ml of 5% solution) for 60 min. Most of the alcohol was then removed by distillation, the residue diluted with water and then acidified. The solid dihydroxy acid was collected, dried, treated with about 10 ml of cold petroleum ether (40–60° C), filtered and crystallised from alcohol or ethyl acetate.

When oleic acid was first mixed with silver succinate in dry benzene and then refluxed with the gradual addition of iodine, the yield of dihydroxy acid finally was considerably lower.

Further work in this series is in progress. The authors thank Sri. A. N. Menon for some preliminary work and Prof. N. S. Panchanathan for his active interest.

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1. Prevost, C., *Compt. Rend.*, 1933, 196, 1129; 197, 1661.
2. Witcoff, H. and Miller, S. E., *J. Am. Chem. Soc.*, 1947, 69, 3138.
3. Raman, P. S., *Proc. Indian Acad. Sci.*, 1956, 44, 321.
4. Gunstone, F. D., *An Introduction to the Chemistry of Fats and Fatty Acids*, Chapman & Hall Ltd., London, 1958.

2-METHOXY-3-NITRO-5-CHLORO-BENZOHYDRAZIDE—A NEW BENZOHYDRAZIDE FOR CHARACTERISATION OF CARBONYL COMPOUNDS

SEVERAL new substituted benzohydrazides have been described as prospective reagents for characterisation of carbonyl compounds[1]. Haksar and Sethi[2–6] have prepared some mono- and disubstituted ortho-methoxy benzohydrazides. We have further extended this work[7] and now we find that 2-methoxy-3-nitro-5-chlorobenzohydrazide is fairly reactive towards carbonyl groups although it could not yield the derivative with formaldehyde. This was prepared by the chlorination of salicylic acid with concentrated hydrochloric acid and hydrogen peroxide[8] yielding 5-chloro-salicylic acid, methyl 2-methoxy-5-chlorobenzoate[9] was prepared and then converted by nitration[10] into methyl 2-methoxy-3-nitro-5-chlorobenzoate (m.p. 64° C). This nitrated compound was then converted into the corresponding hydrazide (m.p. 278, decomp.) by the usual method employing 60% hydrazine hydrate.

RESULTS

Sl. No.	Carbonyl compound		Molecular formula of hydrazones	Melting point (° C)	% Chlorine	
					Found	Required
1.	Acetone	..	$C_{11}H_{12}O_4N_3Cl$	288 (Decomp.)	12·56	12·43
2.	Acetophenone	..	$C_{16}H_{14}O_4N_3Cl$	290 (Decomp.)	10·65	10·21
3.	Ethyl Methyl Ketone	..	$C_{12}H_{14}O_4N_3Cl$	284 (Decomp.)	11·65	11·85
4.	Salicylaldehyde	..	$C_{15}H_{12}O_5N_3Cl$	286 (Decomp.)	9·51	10·15
5.	Acetaldehyde	..	$C_{10}H_{10}O_4N_3Cl$	282 (Decomp.)	13·59	13·07
6.	*p*-Dimethyl amino benzaldehyde	..	$C_{17}H_{17}O_5N_4Cl$	272 (Decomp.)	8·89	9·42

For preparing the hydrazones, the hydrazide (0·4 gm) was dissolved in ethanol (70 ml) with gentle warming. The carbonyl compound (0·4 gm) was added to the warm solution of the hydrazide and refluxed for 30 minutes, on a hot water-bath. On cooling to 0° C, coloured crystalline hydrazones were obtained, these were filtered at the pump, washed with small quantity of alcohol and recrystallised from either methanol or ethanol.

Formaldehyde did not react with methyl 2-methoxy-3-nitro-5-chlorobenzohydrazide to give hydrazones. A drop of hydrochloric acid was also added to the reaction mixture to catalyse the hydrazone formation but with no avail.

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School of P.G. Studies,
Jiwaji University,
Gwalior, *June* 18, 1976.

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C. N. HAKSAR.

1. Haksar, C. N. and, Wankhade, R. D., "New reagents for carbonyl compounds," *Labdev. J. Sci.*, 1965, 3 (4).
2. — and Sethi, R. K., "Some new reagents for carbonyl compounds, *Labdev. J. Sci. Technol.*, 1971, Part I, **9A** (1), 40.
3. — and —, *Ibid.*, 1971, Part II, **9A** (2), 102.
4. — and —, *Ibid.*, 1971, Part III, 9 A (3–4), 158.
5. — and —, *Ibid.*, 1971, Part IV, **9A** (3–4), 205.
6. — and —, *Ibid.*, 1972, Part V, **10A** (1), 48.
7. — and Thakkar, N., under publication in *Jiwaji University Research Journal.*
8. Leulier, A. and Pinet, L., *Bull. Soc. Chim.*, 1927, **41**, 1362; *Chem. Abstr.*, 1928, **22**, 5342.
9. Sachs, F. and Harold, V., *Ber. dt. Chem. Ges.* 1907, **40**, 2218.
10. Hirsch, *Ibid.*, 1900, **33**, 3239.

ISOLATION OF THERMOPHILIC FUNGI FROM SOILS*

IMPORTANCE of temperature as one of the envronmental factors affecting the microflora of a habitat qualitatively and quantitatively has been well documented[2-4,7]. Although its direct influence on the prevalence of fungi has been worked out, its usage as a technique indirectly to isolate thermophilic and thermotolerant fungi has been confined to a few habitats such as composts, bird's nests and other self-heating materials[1,5,6,8-10,12]. The present report deals with the isolation of such fungi from rice-cultivated soils around Madras.

Soil samples from two rice-fields were analysed for the flora using dilution and soil plate methods. Poured plates were kept in desiccators and incubated at 45°, 55° and 60° C. Three replicates were maintained for each sample and the fungal count made on the seventh day. Martin's rose Bengal agar and Emerson's Yeast extract-phosphate-starch-sulphate (YpSs) agar were used in this study. Pure cultures were maintained and the fungi identified according to Cooney and Emerson[3].

Table I shows the species of thermophilic fungi isolated from the soils by the two techniques. Of the ten species isolated, dilution plate method registered six species while soil plate nine, thereby showing the efficiency of the latter. Also, *Aspergillus fumigatus* has consistently been isolated from both the soil samples, irrespective of the increase in the incubation temperature. Only one species viz., *Torula thermophila* was isolated by soil-plate method at the incubation temperature of 55° C whereas others occurred at 45° C. No fungus was recorded at 60° C.

Thermophilism, initiated by Miehe[9], has been studied by different investigators in detail to understand mycofloras of diversified habitats. Maheswari

TABLE I

Thermophilic fungi isolated from soils

Fungi	Techniques	
	Dilution plate	Soil plate
Phycomycetes :		
Mucor pusillus Lindt.	−	+
Ascomycetes :		
Thermoascus aurantiacus Miehe	+	+
Chaetomium thermophile var. *thermophile* La Touche	−	+
C. thermophile var. *dissitum* La Touche	+	+
Deuteromycetes :		
Aspergillus fumigatus Fres.	+	+
Humicola grisea var. *thermoidea* Traaen	+	+
H. insolens Cooney and Emerson	−	+
H. lanuginosa (Griffin and Maublank) Bunce	+	−
Torula thermophila Cooney and Emerson	−	+
Malbranchea pulchella var, *sulphurea* Miehe	+	+
Total	6	9

while working with composts, dung and other sources for isolating thermophilic fungi emphasized that work on different soil samples would greatly enhance the validity of this indirect method of isolating fungi that are dependent upon a specific environmental factor, *i.e.*, temperature. Present work has indeed shown that this selective method is useful in isolating fungi quite varied from the normal flora isolated by incubation at room temperature[11], suggesting that this method could, by itself, be used as a technique to isolate fungi of specific group from soils that aid in the decomposition of cellulose and other organic matter in soil along with other common cellulolytic fungi.

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1. Apinis, A. E. and Pugh, G. J. F., *Mycopath. Mycol. Appl.*, 1967, 33, 1.
2. Bhuvaneswari, K., *Ph.D. Thesis*, University of Madras, 1958.
3. Cooney, D. G. and Emerson, R., *Thermophilic Fungi*, Academic Press, N.Y., 1969.
4. Cowling, E. B. and Kelman, A., *Phytopathology*, 1964, 54, 373.
5. Crisan, E. V., *Contrib. Boyce Thompson Inst. Pl. Res.*, 1964, 22, 291.
6. Fergus, C. L., *Mycologia*, 1964, 56, 267.
7. Greaves, J. E. and Jones, L. W., *Soil Sci.*, 1944, 5, 377.
8. Maheshwari, R., *Curr. Sci.*, 1968, 37, 277.
9. Miehe, H., *Die Selbsterhitzung Des Heus. Eine Biologische Studie.*, Publ. Gutav Fischer, Jena., 1907, p. 127.
10. Ramabhadran, V., *Proc. 1st All India Symp. Agric. Microbiol.*, Univ. Agric. Sci., Bangalore, 1968, p. 44.
11. Sundaram, B. M., *Ph.D. Thesis*, University of Madras, 1969.
12. Warcup, J. H., *Trans. Br. Mycol. Soc.*, 1951, 34, 515.

NITROGEN FIXATION BY A SALT AND ALKALI TOLERANT STRAIN OF *AZOTOBACTER CHROOCOCCUM*

IN earlier studies on asymbiotic nitrogen fixation in calcareous soil of Pusa (North–Bihar), a positive significant correlation between *Azotobacter* population and the amount of nitrogen fixed in different types of soils was observed (Rai *et al.*, 1975)[4]. Soil properties including soluble salts were also found to influence microbial population in these soils (Rai *et al.*, 1972–73)[3]. Moreover, the *Azotobacter* population in calcareous saline-alkali soils of North–Bihar, covering several districts has been found to be relatively depressed (Anon., 1975)[1].

For augmenting soil nitrogen in saline and alkali soils, a strain of *Azotobacter chroococcum* growing at pH 9–0 with 0·2% salt (NaCl) was isolated from calcareous saline-alkali soil of Pusa Farm, by enrichment technique. Its nitrogen fixing capacity was determined in Ashby's mannite solution at different pH with and without added salt (Table I). The culture was tested for enrichment of N in soils of varying characteristics (Table II) and the gain of N-fixed was determined.

A perusal of the data indicated that the culture was effective during a wide pH range (6·2 to 8·8). It was capable of tolerating salinity ranging from 35 to 195 mg/100 g soil. Similarly it flourished

TABLE I

Gain in N-fixed/g.C in liquid medium at different pH range by Azotobacter chroococcum

Sl. No.	pH range	5·5	6·0	6·5	7·0	7·5	8·0	8·5	9·0	9·5
1.	Gain in N-fixed (*a*) With 0·2% salt ..	5·86	8·30	11·76	12·36	12·40	12·04	12·46	11·76	No growth
2.	Gain in N-fixed (*b*) Without salt ..	5·80	8·35	11·90	12·60	12·30	12·30	12·50	11·90	No growth

TABLE II

Soil characteristics and nitrogen fixation by Azotobacter chroococcum

Sl. No.	Soil texture	Org. C%	pH	T.S.S. mg/100 g soil	$CaCO_3$ %	Amount of N-fixed/g soil* (uninoculated) in mg			N-fixed/g* soil (inoculated) in mg
						Treated	Control	N-gain	
1.	Sandy loam ..	0·745	6·2	70	2·68	5·24	1·42	3·80	6·5
2.	Sandy loam	0·512	7·6	35	0·56	4·80	1·40	3·40	6·2
3.	Sandy loam ..	0·416	8·2	184	32·5	4·56	1·42	3·14	6·7
4.	Sandy loam ..	0·212	8·5	95	44·6	4·38	1·40	2·98	4·7
5.	Silty clay loam ..	0·590	8·8	195	38·0	4·83	1·40	3·43	6·3

* 100 ml Ashby's mannite solution containing one gram soil was incubated.

at varying amounts of $CaCO_3$ (nil to 44·6% of the soil). In liquid medium with 0·2% NaCl, the gain in N-fixed was quite high in the pH range 6·5 to 9·0. It gradually decreased below pH 6·5 and the isolate could not grow either at pH 5–0 or at pH 9·5. Mischustin and Shil'nicova (1971)[2] have also mentioned that *Azotobacter* fails to grow above pH 9·0 and below pH 5·4.

The culture may be tried successfully for enriching soil N in calcareous and non-calcareous soils as well as in the saline-alkali soils.

Sugarcane Research Institute, N. AHMAD.
Pusa, Bihar, M. RAI.
June 26, 1976. B. P. SAHI.

---

1. Anonymous, "The effect of soil microbial population and their bearing on the fertility status," Unpublished data, Sugarcane Res. Institute, Bihar, Pusa, 1975.
2. Mischustin, E. N. and V. K., Shil'nicova, *Biological Fixation of Atmospheric Nitrogen*, The Macmillan Press Ltd., London/Madras, 1971, p. 198.
3. Rai, S. N., Rai, M. and Singh, S. N., *Proc. Bihar Acad. Agril. Sci.*, 1972–73, 20-21, 41.
4. —, — and —, *Curr. Sci.*, 1975, 44 (6), 208.

PERSISTENCE OF TEMIK (ALDICARB) IN SOIL AND ITS RESIDUES IN BHENDI FRUITS (*ABELMOSCHUS ESCULENTUS*)

TEMIK [2-methyl-2-(methylthio) propionaldehyde O-methyl carbamoyloxime] is an effective insecticide against many sap feeding pests of crops. It is mainly applied to soils as 10% granules. The persistence and degradation in soil and the resultant bioactivity may depend on the type of soil, soil moisture, crops grown and other associated factors. Therefore the present investigation was aimed to assess the persistence of aldicarb in clay loam soil planted with bhendi crop and its residues in bhendi fruits.

A field experiment was laid out with soil treatments of 0·5 and 1·0 kg ai/ha of aldicarb applied at the time of sowing of bhendi seeds (Pusa Savani), The field was of clay loam type with a pH of 7·9 and organic matter content of 0·25%. Composite soil samples were collected from each treatment on 1, 15, 30. 45, 60 and 75 days after application. Bhendi fruit samples were collected on 50 and 55 days after sowing. Both soil and fruit samples were analysed for Aldicarb residues (Jhonson and Stansburg, 1966). The Aldicarb content of soil residues are presented in Table I.

TABLE I

Days after application	Residues of Aldicarb (ppm)	
	0·5 kg	1·0 kg
1	1·35	2·12
15	0·26	0·73
30	0·19	0·60
45	0·09	0·42
60	0·05	0·11
75	ND	0·09

The results indicated that the insecticide had persisted in soil up to 60 days for 0·5 kg ai/ha and 75 days for 1·00 kg ai/ha and the levels of residues were 0·05 and 0·09 ppm respectlvely. The degradation in the first fortnight was very rapid and thereafter the dissipation was gradual. Studies conducted by Andrews *et al.* (1971) with S-methyl C^{14} aldicarb also revealed that 0·05 to 0·07 ppm of aldicarb were detected after 90 days of application in sandy loam soil. Bull *et al.* (1970) in their studies with S^{35} aldicarb and highlighted the importance of soil texture and moisture content of soil. There was no difference due to pH but at 100% field capacity moisture level the decomposition to non-toxic products was rapid.

The results of aldicarb residues in bhendi fruits are presented in Table II.

TABLE II

Pickings	Residues in ppm		Tolerarce level in ppm
	0·5 kg	1·0 kg	
I picking (50th day)	0·12	0·17	0·20
II picking (55th day)	0·08	0·13	0·20

The results revealed that for both the doses, the residues in fruits were found to be less than the permissible tolerance level of 0·2 ppm as fixed by the FDA of USA and hence can safely be used for the plant protection work of bhendi crop.

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K. RAJUKKANNU.
R. REGURAJ.
T. R. SUBRAMANIAM.
K. K. KRISHNAMOORTHY.

---

1. Andrewes, N. R., Bagley, W. P. and Herott, R. A., "Fate and carry over properties of temik (aldicarb) pesticide in soil," *J. Agrl. Fd. Chem.*, 1971, 19 (4), 727.
2. Bull, D. L., Stokes, R. A. and Coppedge, J. R., "Further studies of the fate of aldicarb in soil," *J. Econ. Ent.*, 1970, 63, 1283.
3. Jhonson, D. P. and Stansburg, H. A. Jr., "Determination of Temik residues in raw fruits and vegetables," *J. Assoc. Offi. Anal. Chem.*, 1966, 49, 399.

FAILURE OF THE DEOLONE ROAD BRIDGE ON THE SONE RIVER, SHAHDOL DISTRICT, MADHYA PRADESH

THE Deolone road bridge situated on the Sone river, in Shahdol District, Madhya Pradesh, failed on August 22,, 1975, during floods after 25 years of service. It was 230 m. long and 12 m. high multispan bridge which stood on 21 tough cement concrete piers. It broke so badly, that right abutment and adjacent 19 piers have been knocked out. Out of the broken piers, 7 are holding ground, while all the others appear to have succumbed to the scouring action of the flowing water.

The Deolone bridge was aligned across the strike of the country rocks, so that different rock types are met with, 'along its foundation. The main rock-types present are quartzites, shales and limestones, all belonging to the Basal stage of the Semri Series (Lower Vindhyan). They are dipping at 70° to NW. Only 200 m. downstream of the bridge, the Sone river turns abruptly and cuts across the quartzite ridge forming a water-gap. On examination, this water-gap was found to have been formed along a transverse shear-zone[1] in the rock-beds.

The nature of the Sone river course in this area has been studied by the author and it is found that the river is engaged in down-cutting its channel[2]. The thinly bedded shales which exist in the river-bed along the Deolone bridge, are very weak and disintegrate easily into small fragments. It is therefore sensitive to the scouring action of the flowing water. Further, as the shale strata are steeply inclined and disposed parallel to the flow of the water, the rate of erosion has been comparatively rapid. A transverse shear-zone exists in the rock-beds just down stream of the bridge, where the river takes a sharp turn in an effort to follow it. A 24 m. deep pool is dug up by eddying currents in the shear-zone which has supported the bed erosion near the bridge.

The position of the right abutment of the Deolone bridge was critical, because it was placed near the junction of the shale and quartzite, just at the base of the quartzite ridge. Here the strata, whose strike is parallel to the thrust of the river water, are dipping into the river channel. Since displacement easily takes place parallel to the bedding planes, the valley-wall on which the right abutment was sitting had a tendency to slide into the river.

During floods, the pressure of water-currents near the bridge got concentrated towards the right bank, as the quartzite ridge which exists at the right bank does not allow the flood water to spread but confines it into the river channel. As a result, the river-bed is dug asymmetrically, deeper towards the right bank. This has increased the instability of the right abutment of the bridge.

In all cases the scour is intensified during flood periods. Nothing definite has yet been known about the relation between the depth of scour and the rise of flood water above the ordinary level. However, in some experiments the depth of the river bed scoured was found to be about one-third the amount of water surface rise[3]. During the flood in the Sone river which occurred on August 22, 1975, and, which is responsible for the failure of the Deolone bridge, the water surface rose to about 17 m. and therefore the bed erosion must have been quite deep.

Thus it may be concluded that the foundation of the bridge piers which was in shales, had progressivety been eroded by the running water and the foot-hold of the bridge got weaker day by day. On August 22, 1975, when the river was in flood, the bridge lost ground and collapsed into the river. This is a clear cut example, where the bridge failure has occurred as a result of its construction on a geologically defective site.

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---

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1. Bangar, K. M., "Shear discontinuity along the Sone river bed at Bansagar Dam site, South of Rewa, M.P.," *Ind. Geotech. Jour.*, 1974, 4 (2), 188.
2. —, "On some aspects of the part of the Sone river course lying South of Rewa, M.P.," *Quart. Jour. of Geol. Minn. and Met. Soc. Ind.*, 1975, 47 (2), 97.
3. Leopold, L. B., Wolman, M. G. and Miller, J. P., *Fluvial Process in Geomorphology*. Eurasia Publishing House, New Delhi, 1969, p. 230.

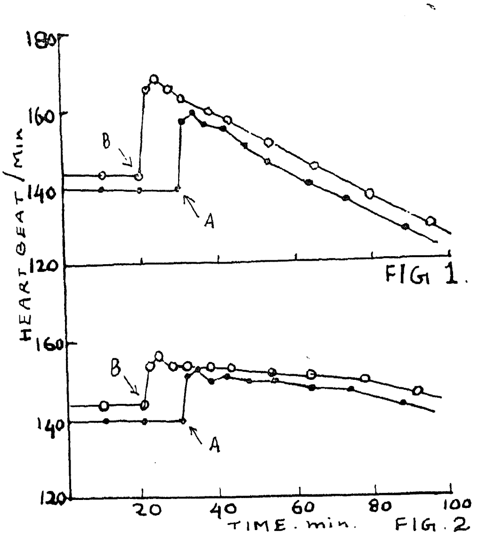
STIMULATORY EFFECT OF THE NEUROACTIVE SUBSTANCE ON ISOLATED COCKROACH HEART

Earlier studies indicated that certain drugs and insecticides increased the frequency of heart beat[1,2]. It has been reported that application of the insecticides is likely to release certain toxins lethal to insects from the nervous system[3,4]. Sudershan and Naidu[5] (1967) working on isolated cockroach heart found that a specific toxin released into cockroach blood by malathion and pyrethrum, increased the heart beat frequency. In the present investigation attempts have been made to isolate the neuroactive substance from endrin treated cockroach blood and to study its effect on the isolated cockroach heart.

Laboratory reared male adult *Periplaneta americana,* L. was used for the experiments. The isolated cockroach heart technique described by Krijgsman *et al.*[1] (1950) was employed. LC_{90} of endrin dissolved in ethanol was injected intraperitoneally to cockroaches. Four hours after the treatment, blood was collected by centrifugation and the neuroactive substance was isolated from blood as described by Sternburg *et al.*[4] (1959). Test solutions were prepared in ethanol (wt/vol) and incorporated into the physiological solution. Neuroactive substance and synergist combinations were made at the ratio of 1 : 1. The concentration of ethanol used was not detrimental to the isolated cockroach heart.

Addition of neuroactive substance ($1 \cdot 6 \times 10^{-6}$ and $3 \cdot 3 \times 10^{-6}$) induced an immediate increase in the heart beat frequency followed by rapid decline (Fig. 1). When it is mixed with piperonyl butoxide ($1 \cdot 4 \times 10^{-6}$ M) and sesamin ($1 \cdot 4 \times 10^{-6}$ M) slight initial stimulation was seen which sustained for some time (Fig. 2).

A detailed study of endrin on isolated cockroach heart[6] reveals that it acts at the cardiac ganglia by paralysing them and acetylcholine is not involved. In combination with the synergists, the effect of endrin is considerably increased thus suggesting synergism. The fact that no apparent change was noticed in the original action of neuroactive substance when mixed with piperonyl butoxide and sesamin, indicates that synergism is absent. The sustained action (Fig. 2) may be due to the effect of synergists on the heart. In the present communication it is difficult to assess whether degradation of neuroactive compound is taking place in the insect body as in the case of endrin. However, an enzyme which destroys biologically active substance has been reported from the blood of cockroach[4]. If it is true, the factor responsible for the degradation of neuroactive substance is probably not interfered with by the synergist.



FIGS. 1–2. Fig. 1. Effect of neuroactive substance (A = 1·6 × 10^{-6}, B = 3·3 × 10^{-6}) on isolated heart of Cockroach. Fig. 2. Effect of neuroactive substance in combination with A : Piperonyl butoxide (1·4 × 10^{-6} M). B : Sesamin (1·4 × 10^{-6} M). Arrows indicate addition of the test solution.

A detailed account on the nature and biological activity of neuroactive substance will be reported elsewhere.

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---

1. Krijgsman, B. J., Dresden, D. and Berger, N. E., *Bull. Ent. Res.*, 1950, 41, 141.
2. Naidu, M. B., *Ibid.*, 1955, 46, 205.
3. Sternburg, J. and Kearns, C. W., *Science*, 1952, 116, 144.
4. —, Chang, S. C. and Kearns, C. W., *J. Econ. Ent.*, 1959, 52, 1070.
5. Sudershan, P. and Naidu, M. B., *Ind. J. Exptl. Biol.*, 1967, 5, 215.
6. Purushotham Rao, A., *Ph.D. Thesis* submitted to Osmania Univ., Hyderabad, India, 1973.

SOLUBILITY OF PROTEINS OF MUSCLE IN FATIGUE

IN isolated muscle fatigue is associated with the accumulation of lactic acid[1] and other metabolic byproducts and metabolism by acidification or otherwise[2,3] which contribute to disturbances of internal equilibria. Local accumulation of lactate might be expected to induce water and electrolyte shift due to osmotic acid-base changes in muscle cells[4-6]. To reduce the acidity and other side effects, the buffering capacities of sarcoplasmic preteins are utilized to safegaurd other structural components[7]. This paper is aimed at analysing the ionization and soluble properties of proteins of muscle in fatigue by salting out processes of various fractions of proteins.

Rana hexadactyla were double pithed and the gastrocnemius muscle of both the legs were Isolated with least injury. They were washed in amphibian ringer[8], several times to recover from shock effects. One of the muscles was immersed in ringer and made to fatigue by giving electrical stimulations of 120 shocks per minute of 10 volts D.C. strength (INCO/CSIO student stimulator, AMBALA) continuously until there was no response. The fatigued muscle was rapidly cooled to 0° C to prevent residual metabolism. The contralateral control muscles were treated in the same way except that they were not stimulated. Total, soluble and insoluble proteins were estimated in the aqueous homogenates of muscle by the method of Knights *et al.*[9] (1962). Alpha, beta and gamma globulin and albumin type of proteins were sedimented from the supernatant fraction of the muscle homogenate, following the procedure of Cohn *et al.* (1940) for serum[10].

The decrease in the levels of soluble proteins is nearly proportional to the increase of insoluble proteins (Table. I). Practically there is no change

TABLE I

Levels of total, insoluble and soluble proteins in muscle expressed as mg/gram wet weight. The values are means of ten individual observations

Muscle	Total	Insoluble	Soluble
Control	164·1 ±14·83	120·2 ±15·29	43·9 ±2·62
Fatigued	163·4 ±15·74	122·3 ±17·45	41·1 ±2·38
% Deviation	− 0·43	+ 1·75	−6·37
* '*t*' test	NS	NS	NS

* NS = Not Significant.

in total protein content. Significant fall in the levels of gamma globulin, alpha beta globulin, and albumin type of proteins is observed on fatigue (Table II). This indicates that solubility of these proteins is more affected during fatigue as it might be particularly involved in buffering action.

TABLE II

Levels of globulins and alubumin type of proteins in muscle expressed as mg/gram wet weight. The values are means of ten individual observations

Muscle	Gamma globulin	Alpha and beta globulin	Albumin
Control	7·78 ±0·53	12·30 ±0·98	16·55 ±1·61
Fatigued	6·43 ±0·43	10·60 ±1·23	13·85 ±1·81
% Deviation	−17·3	−13·8	−16·4
*‘t’ test	0·01 S	0·05 S	0·05 S

* S = Significant.

Alternation in the levels of different types of proteins is not because of the rapid degradation or synthesis since such changes cannot be expected after a short period of extensive work. Changes in the intracellular environment of the muscle affects the ionization of sarcoplasmic proteins[11] and also the buffering capcity of proteins[12]. So it is suggested that variation in different protein fractions is only due to the alteration in ionization and soluble properties because of their involvement in buffering of acid and other metabolic byproducts produced during fatigue to protect the structural components of contractile machinery.

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India, *July* 9, 1976.

---

1. Fletcher, W. M. and Hopkins, F. G., *J. Physiol.*, 1907, 35, 247.
2. Hill, A. V. and Kupalov, P. S., *Proc. Roy. Soc. B*, 1930, 105, 313.
3. Mommaerts, W. F. H. M., In Luisada, *Cardioloy*," Yearbook Publishers, Chicago, 1959, 2, 3.
4. Bergstrom, J., Guarnieri, G. and Hultman, E., *J. Appl. Physiol.*, 1971, 30, 122.
5. Ahlborg, B., Bergstrom, J., Ekelund, L. G. and Hultman, E., *Acta Physiol. Scand.*, 1967, 70, 129.
6. Roth, G., *Clin. Sci.*, 1966, 30, 417.
7. Eckel, R. E., Bortschner, A. W. and Wood, D. H., *Am. J. Physiol.*, 1959, 196, 811.
8. Cavanaugh, G. M., *Formulae and Method* Marine Biological Laboratory, Woodshol Mass., 1956.
9. Knights, E. M., Mcdonald, R. P. and Ploompun, J., *Ultramicromethods for Clinic Laboratories*, Grune and Stratton, Ne York, 1962.
10. Cohn, E. J., McMeckin, T. L., Onclay, J. L Newell, J. M. and Hughes, W. L., *J. A Chem. Soc.*, 1940, 62, 3386.
11. Indira, P. and Swami, K. S., *Indian J. Exp Biol.*, 1965, 3, 5.
12. Kumudavalli, I. and Swami, K. S., *Ibid.*, 196 5, 162.

GYNOPHORE NUTRITION IN GROUNDNUT

THE importance of Calcium in pod developm is well known and direct absorption by developi fruits when calcium is applied in the peg regi has been reported by Shibuya and Suzhuk Bolhuis and Stubbs[2] and Seshadri[3]. But the p sence of calcium at root region is not equally eff tive. The difference in ion uptake by pegs a roots deserves detailed study. An interesti observation by Pal and Laloraya[4] has indica that root level sodium does not interfere wi Calcium uptake. But calcium uptake by the develo ing pods is inhibited by sodium. The utility terms of nutrient availability is obviously co ditioned by the physico-chemical properties of t membranes of the root and the peg.

In the present investigations involving a provi of single and multiple deficiency conditions the peg zone with complete nutrition at root zo sand culture studies employing Arnon and Ho land's[5] nutrient solution were made (K–390, C 120, Mg–48, NO_3N–224, NH_4–N–28, P–62 a S–64 ppm). TMV 2 bunch strain was used f the study. The gynophore regions were separa from the root zone by a plastic container. T technique adopted comprised insertion of the ro of the young groundnut plant through a glass tu fitted to the plastic container. The root develop in the pot containing sand that was irrigated wi complete nutrient solution. The junction of t glass tube at the base of the plastic container w sealed by wax. The plastic container was fill with washed sand irrigated with Arnon and Ho land culture solution so modified as to prov single and multiple deficiencies (Table II). Chem cal analysis of shell for nitrogen[6], phosphoru potassium and sodium[8] and for calcium[9] a magnesium was made. Oil content of kern (Soxhlet[10]) was determined.

Plants were harvested from five replica Yield of dry pods (g/plant) was determined the data are presented (Table I).

TABLE I

Yield of dry pods (g/plant) as influenced by single and multiple deficiencies at peg zone

Treatment	Yield	Treatment	Yield	Treatment	Yield
(1) Complete	7·21	(6) — N — K	6·10	(11) — Ca — Mg	3·50
(2) — N	2·51	(7) — N — Ca	1·90	(12) — N — K — Ca	1·90
(3) — K	4·00	(8) — N — Mg	5·10	(13) — N — K — Mg	3·50
(4) — Ca	2·90	(9) — K — Ca	5·50	(14) — N — Ca — Mg	5·50
(5) — Mg	6·50	(10) — K — Mg	1·10	(15) — K — Ca — Mg	1·00
				(16) — N — K — Ca — Mg	2·00

C.D. (P = ·01) – 2·069.

TABLE II

Chemical composition of shell (mg/g) (Groundnut TMV 2) and oil content (per cent) of kernels as influenced by single and multiple nutrient deficiencies at peg zone

Nutritional Status	Nutrient element in shell (mg/g)					Oil content per cent
	N	P	K	Ca	Mg	
Complete ..	5·6	5·0	43·1	2·9	·48	49·8
Minus N ..	5·6	4·9	27·0	1·9	·38	32·7
Minus K ..	4·2	3·1	10·6	2·9	·29	41·0
Minus Ca ..	4·2	0·7	13·2	1·1	·29	41·0
Minus Mg ..	9·8	2·8	35·4	1·8	·19	44·3
Minus N and K ..	4·2	2·0	21·6	1·8	·09	33·3
Minus N and Ca ..	4·2	2·4	16·2	1·1	·19	38·5
Minus N and Mg ..	5·6	2·3	19·2	1·9	·18	42·2
Minus K and Ca ..	7·0	3·2	17·4	1·1	·38	44·5
Minus K and Mg ..	7·0	2·7	21·6	1·1	·19	46·5
Minus Ca and Mg ..	4·0	4·9	35·2	1·9	·29	38·0
Minus N K Ca ..	4·2	1·9	5·4	2·6	·19	33·0
Minus N K Mg ..	5·6	2·0	13·3	2·6	·09	38·6
Minus N Ca Mg ..	4·2	1·9	6·0	1·8	·19	32·5
Minus K Ca Mg ..	7·0	4·2	26·2	1·8	·38	42·2
Minus N K Ca Mg ..	4·2	6·0	10·2	1·4	·09	33·3

A deficiency of nitrogen reduces yield by 65·2% and an equal degree of severity is met with in calcium deficiency decreasing the yield by 59·8%. Multiple deficiencies show more depression by 73·6 and 84·7% due to –N–Ca and –K–Mg combinations respectively. Imposition of additional deficiencies over those of N and Ca has negligible yield depression but imposition of calcium deficiency over those of K and Mg effect further depression by 86·1%. This trend is interesting by way of highlighting the limiting influence of calcium (Bledsoe *et al.*[11] and Gopalakrishnan and Nagarajan[12]). This situation influencing the nutritional status in peg zone is more significant compared to a similar status at the root zone.

Chemical composition of shell (Table II) shows no reduction in nitrogen content under nitrogen deficiency. Under other circumstances of multiple deficiencies, nitrogen content is increased. Magnesium deficiency increases N content of shell phosphorus content is acutely depressed due to calcium deficiency (5·0 *Vs* 0·7). Phosphorus content is very much lowered under any deficiency. A deficiency of N, Ca and Mg tends to affect K content very acutely (43·1 *Vs* 6·0) even as a combined deficiency of K and Ca or K and Mg affects Mg content (0·48 *Vs* 0·09).

Any nutrient deficiency impairs oil content. Magnesium deficiency in conjuction with N and Ca or K brings down oil content to 32·5 and 46·5% respectively compared to 49·8% in the control.

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---

1. Shibuya, T. and Suzhuki., *Proc. Crop. Sci. Soc., Japan.,* 1955, 23, 87.
2. Bolhuis, G. G. and Stubbs, R. W., *Netherlands Jour. Agri. Sci.,* 1955, 3, 220.
3. Seshadri, C. R., *The Indian Central Oilseeds Committee,* Rev. Theodore A. Pereira at the Examiner Press, Fort, Bombay, 1962, p, 1.
4. Pal, R. N. and Laloraya, M. M., *Experentia.,* 1967, 23, 382.
5. Arnon, D. I. and Hoagland, D. R., *Soil Sci.,* 1940, 50, 463.
6. Humpheries, E. C.. *Modern Methods of Plant Analysis,* 1956, p. 468.
7. Jackson, M. L., *Soil Chemical Analysis,* Constable and Co. Ltd., London, 1962, p. 498.
8. A. O. A. C., *Official Methods of Analysis of the Association of Agricultural Chemists,* Washington, D.C. 1960.
9. Sankaran, A., *Laboratory Manual for Agricultural Chemistry,* Asia Publishing House, Madras, 1966, p. 57.
10. —, *Ibid.,* 258.
11. Bledsoe, R. W., Comar, C. L. and Harris, H. C., *Science,* 1949, 109, 329.
12. Gopalakrishnan, S. and Nagarajan, S. S., *Indian Oilseeds Jour.,* 1958, 2, 5.

EXUDATION OF PHOSPHORUS (^{32}P) FROM ROOTS OF COFFEE PLANTS

EXUDATION of foliar applied radioactive phosphorus (^{32}P) through roots of eight month old plants was more in robusta (*Coffea canephora* Pierre. cv. S. 274) than in arabica (*Coffea arabica* L. cv. S. 795)[1]. The effect of the plant age on the exudation of ^{32}P is described in this note.

Seedlings of the two coffee species were raised following the technique described previously[1]. The seedlings were then transferred to plastic pots (25 × 25 cm) and earthenware (lined with thick polythene sheet) crocks (30 × 38 cm) filled with weighed quantities of a mixture of jungle soil + farmyard manure + sand (6 : 2 : 1), and grown under pandal shade upto 25 and 50 months. For 25 month old plants, 1·25 g of labelled (^{32}P) superphosphate (supplied by BARC). dissolved in 50 ml water (adjusted to pH 6·5 with lime water) was sprayed on the foliage of each plant, the soil surface being covered with a thick polythene sheet. In the case of 50 month old plants, the leaves on only two secondary branches (with equal number of leaves) were sprayed with 1·25 g of labelled (^{32}P) superphosphate dissolved in 30 ml water. taking all the other precautions as before. In both the experiments, 48 hr. after feeding the radioisotope, the plants were depotted. A weighed quantity of the well mixed soil sample was died under infrared heat and the radioactivity monitored in a G.M. counter. The quantity of ^{32}P exuded through roots was calculated per plant. There were three replications for each coffee species.

With increase in the age of the plants from 25 to 50 months, the volume of the root system also increased and the exudation of P was also more in both the species (Table I). However, in both the age groups, roots of robusta plants exuded more radioactive phosphorus than arabica, in the 48 hr. period. The present data confirm the earlier findings[1,2,3].

TABLE I

Exudation of foliar applied phosphate (^{32}P) through root (per plant) of two age groups of coffee plants

Coffee species	% of applied activity exuded		Quantity of ^{32}P exuded (mg of P)	
	25 months	50 months	25 months	50 months
Arabica	0·003	0·020	0·6	3·9
Robusta	0·005	0·041	1·1	8·3

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---

1. Balasubramanian, A. and Gopal, N. H., *Curr. Sci.*, 1975, 44, 553.
2. Gopal, N. H. and Balasubramanian, A., *Indian Coffee*, 1975, 39, 58.
3. — and D'Souza, G. I., *Ibid.*, 1975, 39, 139.

CHANGES IN OIL AND FATTY ACID COMPOSITION OF LINESS (*LINUM USITATISIMUM* L.) UNDER VARYING PHOTOPERIODS

In an earlier communication, it has been shown that change in photoperiod from 14 to 19 hours resulted in the increase in oil content and the degree of unsaturation in flax seed[1]. The experiment describes the effect of long photoperiods on oil and fatty acid composition in a flax variety morphologically different from the cultivated ones in India and grown particularly for fibre. The present note describes the effect of short and long photoperiods on oil and fatty acid composition in an Indian linseed cultivar.

Seeds (var. SH–1) were sown in pots with 20 replicates. Seedlings (7 day old) were shifted to three photoperiodic conditions *i.e.*, short photoperiod (8 hr. exposures to natural light), normal photoperiod (natural day) and long photoperiod (24 hr. light, consisting of natural light supplemented with 100 watt incandescent filament lamp during the night). Observations on emergence of flower bud and seed weight per plant were recorded. The oil in the seed was determined following cold percolation method[2]. The fatty acid composition was determined on duel column gas liquid chromatograph Shimadju Model GC 4 BPTF in methylated samples[3].

It is observed from Table I that long photoperiodic treatment hastened flower bud emergence whereas short photoperiod had the opposite effect. Seed weight per plant was reduced under both the photoperiodic treatments (short or long). The oil content was not affeced by long photoperiodic treatment; on the other hand it decreased considerably when plants were exposed to short photoperiod. Likewise the degree of unsaturation did not differ from the normal photoperiod under the long photoperiodic treatment. Under short photoperiodic treatment however, an increase in oleic acid and a decrease in the linolenic acid were observed.

TABLE I

Effect of photoperiod on flower bud emergence, seed weight, oil content and fatty acid composition in linseed (var. SH–1)

Treatment	Short photoperiod	Normal photoperiod	Long photoperiod	CD at 5%
Days to flower bud emergeuce	75·0	48·0	39·6	1·54
Seed weight per plant (g)	1·6	2·6	1·8	0·044
Oil content (g) percent dry weight	38·6 ±0·1	40·3 ±0·2	40·8 ±0·2	..
Fatty acid composition:				
Palmitic	6·6	6·6	5·8	..
Stearic	8·4	6·1	6·7	..
Oleic	38·0	30·7	30·0	..
Linolic	15·9	12·6	12·0	..
Linolenic	31·1	44·5	45·6	..

It has been suggested that oleic acid serves as the precursor of linolenic acid[4].

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---

1. Sosulski, F. W. and Gore, R. F., *Can. J. Pl. Sci.*, 1964, 44, 381.
2. Kartha, A. R. S. and Sethi, A. S., *Ind. J. Agr. Sci.*, 1957, 27 L, 216.
3. Craig, B. M. and Murty, N. L., *Am. Oil Chem. Soc.*, 1959, 36, 549.
4. Canvin, D. T., *Can. J. Pl. Sci.*, 1965, 43, 71.

PHYSIOLOGY OF BHENDI PLANTS, *ABELMOSCHUS ESCULENTUS* (L.) ASSOCIATED WITH ITS PREFERENCE AND FEEDING BY THE REDSPIDER MITE *TETRANYCHUS CINNABARINUS* BOISDUVAL

PALANISAMY[1] studied several varieties to mite infestation under glass house as well as in field and screened some less preferred types. In this study a detailed account of the nutritive conditions of the plants in relation to the development of the mite as well as on the bio-chemical changes that take place due to its feeding has been reported.

The fecundity rate, total number of larvae hatched out per pair, duration of immature stages, number of days taken by the hatched out larvae to attain adult and longevity of adults were recorded.

Leaf samples collected (8 am) from healthy and mite-infested plants of four varities (Table I) of comparable ages were analysed for moisture, carbohydrates[2], total nitrogen[3], minerals like phosphorus calcium and potassium[4], and resin[5] and free amino acids.

TABLE I

Preference of red spider mites to bhendi varieties (Means of 5 observations)

	Preferred		Non-preferred		C.D. (P = 0·0
	A.E. 75	Pusa Sawani	A.E. 1	Long Green	
1. Fecundity/mite ..	27·2	26·2	18·4	16·3	1·84
2. Duration of immature stages (Days) ..	9·4	9·4	13·6	15·8	0·4
3. Larvae becoming adults*	56·8 (59·6)	58·5 (72·5)	41·8 (44·4)	43·8 (48·0)	 (9·1)
4. Adult longevity in days					
(i) Male	5·1	6·1	2·9	3·1	..
(ii) Female	8·1	7·5	4·5	4·1	..
(iii) Mean	6·6	6·3	3·7	3·6	0·8

* Per cent of larvae becoming adults (P); in parentheses, the values of arc sin $\sqrt{P}$.

Enhanced fecundity rate, increase in per cent of larvae becoming adults, shortening of developmental time of immature stages and increase in longevity of adults (Table I) on varieties A.E. 75 and Pusa Sawani might be the reason for the high population of mites observed by Planisamy[1] on these varieties. These varieties had less of carbohydrates and more of total nitrogen leading to low carbohydrate/nitrogen (C/N) ratio. That low C/N ratio associated with the preference of mite[6] was also reported earlier. Mites were reported to avoid high concentration of carbohydrate in plants by Henneberry[7]. The mite preference for high nitrogen content was reported in many instances and the present findings is in conformity with that made for *Panonychus ulmi*[8], *Tetranychus telarius*[9-14] and *Aceria scheldoni*[15] (Ewing).

Amino acid content was also high in A.E. 7 and Pusa Sawani. The increase in amino acid con- tent having high chemotactic influence on mit had already been reported by Sternlicht *et al.* Out of the six amino acids listed by Mittler[16] th strongly enhance the feeding of aphids, tryptoph was found exceptionally in high amounts and a paragine was found only in these varieties. Furth asparagine, aspartic acid and glutamic acid th enhanced the egg production of *Tetranychus tel- rius*[17] were found in high quantities in prefer varieties. The amino acid tryptophan is the pr cursor for auxin like IAA. The plant parts havi high auxin content were preferred by mites[18] a enable better reproduction and shorter developm period[19]. The high moisture, low calcium and re contents might have facilitated easy penetration cheliceral styles of mites due to more succul nature of the leaves. Low potassium content w found to be associated with the preference of mi in the present study which is in confirmation wi that made by Henneberry and Smith[20], Mathy and Sternlicht *et al.*[15]. The phosphorus content the plant was not found to influence the preferen of varieties to the mites as had been reported earli in the case of *Panonychus ulmi*[14].

Mite feeding had resulted in the reduction carbohydrates and moisture. This might be d to hindrance of photosynthetic activity, as the lea become 'chlorotic' or removal by mite feedi Rajagopal *et al.*[6] observed mite infestation reduc the chlorophyll content. The efficiency of pho synthesis in plants is affected when the affinity chlorophyll for water is interfered with[21]. reduction in carbohydrate synthesis might h

TABLE II

Carbohydrate, nitrogen, moisture, resin, mineral make up and amino acid content of healthy and mite infested leaves of bhendi varieties (Per cent on dry weight basis—Means of three observations)

Sl. No.		Preferred				Non-preferred			
		A.E. 75		Pusa Sawani		A.E. 1		Long Green	
		Healthy	Infested	Healthy	Infested	Healthy	Infested	Healthy	Infested
1. Carbohydrate	..	8·8	8·6	9·1	9·0	10·8	9·8	10·0	9·3
2. Total Nitrogen	..	4·8	5·0	4·3	4·6	4·0	4·2	3·6	3·9
3. Moisture	..	85·3	70·4	87·4	76·5	83·0	74·3	74·7	72·6
4. Resin	..	18·4	12·6	17·8	13·9	22·2	16·6	22·8	17·9
5. Phosphorus (P_2O_5)	..	1·04	0·79	0·98	0·90	1·12	0·88	1·06	0·74
6. Calcium (CaO)	..	2·72	1·42	2·40	0·98	3·04	2·02	3·24	2·24
7. Potassium (K_2O)	..	3·0	3·4	3·1	3·3	4·2	4·7	4·2	4·4
8. *Amino Acids* (μg/1·0 g fresh tissue)									
(i) Asparagine	..	20	60	Trace	Trace	Trace	Trace	Trace	Trace
(ii) Leucines	..	10	..	10	40	35	30	70	90
(iii) Alanine	..	330	..	640	120	280	200	Trace	Trace
(iv) Glycine	..	Trace	40	Trace	Trace	..	Trace	..	Trace
(v) Aspartic acid	..	500	240	320	70	120	130	140	200
(vi) Glutamic acid	..	350	420	80	Trace	Trace	Trace	..	..
(vii) Histidine and Lysine	..	Trace	170	Trace	180	..	Trace	30	10
(viii) Tryptophan	..	1250	700	460	360	60	80	40	35
Total	..	2160	1630	1510	770	495	440	280	335

resulted in low resin content which is nothing but a polysaccharide. Utilization of amino acids and phosphorus content by mite might have resulted in their reduction. The peculiar feature was that potassium content was increased due to mite feeding. The reason for this increase is not known. However the increase in potassium would have increased the protein synthesis as reported by Chapman[22]. And this increase in protein synthesis might be the reason for high total nitrogen observed in infested leaves.

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---

1. Palanisamy, S., *Studies on the Two-spotted Spider Mite* Tetranychus tilarius *Linnaeus (Acarina: Tetranychidae) on Okra* (Abelmoschus esculentus *Moench*). M.Sc. (Ag.) Dissertation, Madras University, 1971.
2. Somogyi, M., *J. Biol. Chem.*, 1952, 200, 145.
3. Humphries, E. C., *Modern Methods of Plant Analysis*, Vol. I (Springer-Verlag, Berlin), 1956.
4. Jackson, M. L., *Soil Chemical Analysis*, Constable and Co. Ltd., London, 1962.
5. Shewan, J. H., *J. Agric. Sci.*, 1938, 28, 324.
6. Rajagopal, K., Jayaraj, S. and Subramaniam, T. R., *Indian J. Exp. Biol.*, 1970, 8, 44.
7. Henneberry, T. J., *J. Econ. Ent.*, 1962, 55, 134.
8. Chouboussou, F., Delmas, J. and Dureau, P., *C.r. Hebd. Seanc. Acad. Sci. Paris*, 1961, 24, 38.
9. Rodriguez, J. G., Chen, H. H. and Smith, W. T., *J. Econ. Ent.*, 1969, 53, 487.
10. Henneberry, T. J., *Ibid.*, 1962, 55, 134.
11. —, *Ibid.*, 1962, 55, 799.
12. Watson, T. F., *Hilgardia*, 1964, 35, 273.
13. Lall, B. S., Dutta, C. P. and Jayaswal, A. P., *Indian J. Ent.*, 1965, 27, 389.
14. Mathys, G., Baggiolini, M. and Stahl, J., *Entomophaga*, 1968, 13, 357.
15. Sternlicht, M., Regev, S. and Goldenberg, S., *Bull. Ent. Res.*, 1975, 65, 4.
16. Mittler, T. E., *Ent. Exp. Appl.*, 1967, 60, 87.
17. Fritzche, R., *Biol. Zbl.*, 1960, 79, 521.
18. Sternlicht, M., *Israel J. Agric. Res.*, 1967, 17, 57.
19. —, *Ann. Appl. Biol.*, 1970, 65. 221.
20. Henneberry, T. J. and Smith, F. F., *Trans. 11th Int. Congr. Ent. Vienna*, 1960, 2, 49.
21. Gaffron, H., Cited in *Plant Physiology—A Treatise*, Vol. I, edited by F. C. Steward, Academic Press, Inc., New York, 1960, p. 3.
22. Chapman, H. D., *The Citrus Industry*, Vol. I, California Univ. Press, Berkeley, 1968,

DICTYUCHUS ANOMALOUS (NAGAI), A NEW PATHOGEN OF FRESH WATER TELEOSTS

DURING the course of an investigation on fungi associated with fish diseases, fungal infections on adult individuals of *Channa punctatus* (Bloch) were observed during February–March 1975, which usually resulted in the death of the infected hosts. The infected fishes showed the presence of white cottony patches scattered on their body (Fig. 1).

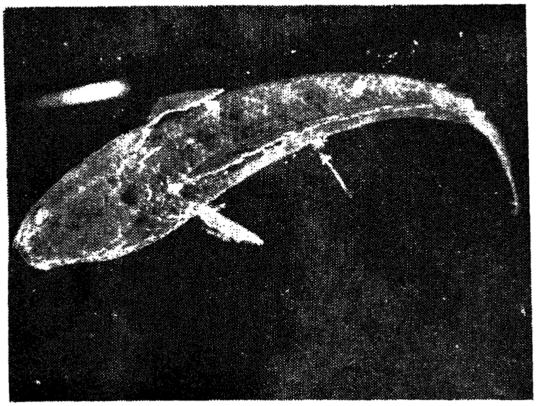

FIG. 1. *Channa punctatus*, showing the infection of Dictyuchus anomalous (White cottony patches on the body).

TABLE I

Controlled laboratory studies demonstrating the infectious ability of Dictyuchus anomalous, *on wounded and unwounded test fishes*

Name and Number of fish		Mycosis evident and death occurred	Mycosis evident within days	Death occurred in days
Wounded:				
Puntius sophore	2	2	7 days	8 days
Colisa lalia	2	2	7 days	8 days
Unwouded:				
Puntius sophore	2	..	..	..
Colisa lalia	2	..	..	..

The pathogen was isolated on boiled hempseed halves in sterile distilled water and its unifungal bacteria free cultures were prepared on the lines described by Johnson[1], Raper[2] and Tiffney[3]. The fungus was identified as *Dictyuchus anomalous* (Nagai).

In order to establish the parasitic ability of the isolate, laboratory inoculation experiments were conducted under controlled conditions, on the lines of Scott and O'Warren[4], using *Puntius sophore* (Hamilton) and *Colisa lalia* (Hamilton) as test fishes.

Hyphae of the parasite were observed growing from the injured areas of the test fishes on 7th day of placing the fish in the infection troughs. The infected fishes died within 24 hours of the appearance of the fungal tufts on the body of the fish. It was further observed that injury greatly lowered the resistance of the fish to the fungal infection (Table I).

Dictyuchus anomalous is reported for the first time as a fish pathogen. *Channa punctatus* is a new host record for the species of *Dictyuchus*.

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---

1. Johnson, T. W. Jr., *The Genus* Achlya : *Morphology and Taxonomy*, Univ. of Michigan Press, Ann. Arbor, Michigan. 1956.
2. Raper, J. R., *Sci.;* 1937. **85**; 342.
3. Tiffney, W. N., *J. Elisha Mitchell Sci Soc.*, 1939 *b*, 55, 134.
4. Scott, W. W. and O'Warren, C., *Tech. Bull. Vir. Polytech. Inst. Virginia,* 1964, 171, 1.

EFFECT OF ORGANIC AND MINERAL FERTILIZERS ON *AZOTOBACTER* IN FLOODED RICE FIELD

ALTHOUGH occurrence and distribution of *Azotobacter* in paddy soils have been investigated[1,6-8], little information is available on the effect of organic and mineral fertilizers on *Azotobacter* population under flooded field conditions. In the present report the effect of organic and mineral fertilizers on the population of *Azotobacter* in the rice field has been discussed.

Soil samples were collected from paddy fields under different treatments—(*i*) control, (*ii*) 100 kg N/ha, (*iii*) rice straw 5 tons/ha, (*iv*) rice straw + 100 kg N/ha, (*v*) rice straw 10 tons/ha and (*vi*) rice straw + 100 kg N/ha. *Azotobacter* population in soils was determined by serial dilution technique on nitrogen-free medium containing (g/l) K_2HPO_4, 1·74; KH_2PO_4, 0·91; $MgSO_4.7H_2O$, 0·30; $CaCl_2.6H_2O$, 0·10; NaCl, 0·50; $FeCl_3.6H_2O$.

0·01; $CaCO_3$, 3·0; agar, 15·0 and glucose, 10·0 and 1 ml of micro-elements.

Organic matter addition at two levels (5 and 10 tons/ha) significantly enhanced the population of *Azotobacter* in a flooded soil (Fig. 1). Moreover, combined application of rice straw at 5 tons/ha with mineral fertilizer further enhanced the *Azotobacter* multiplication. However, combined application of rice straw and mineral fertilizer appeared to be inhibitory when rice straw level was raised to 10 tons/ha.

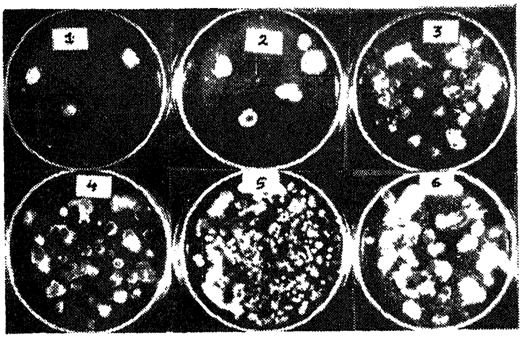

FIG. 1. *Azotobacter* population in flooded soils amended with rice straw and mineral fertilizers. (1) Control, (2) + 100 kg N/ha, (3) rice straw 5 tons/ha, (4) rice straw 5 tons + 100 kg N/ha, (5) rice straw 10 tons/ha, (6) rice straw 10 tons + 100 kg N/ha. (Serial dilution 10^{-3} was used for all soils.)

Independent of the treatment, in all cases, *Azotobacter* was found in greater numbers in the surface layer (0–5 cm) of the flooded soil. The *Azotobacter* population varied from 5–15 × 10^3, 15–60 × 10^3 and 2–25 × 10^3 g^{-1} soil in control, rice straw-amended and mineral fertilizer-treated soils, respectively, during the growing period of rice in the surface soil layer. Individual isolates from different treatments did not differ significantly in their nitrogen-fixing activity (10–12 mg N fixed g^{-1} glucose utilized) as they did in population density.

Azotobacter is found in the oxidized surface layer of a flooded soil and its absence on the roots has been reported[5]. Nitrogen fixation was stimulated when humus compounds were added to medium inoculated with *Azotobacter*[3] and also abundant growth of *Azotobacter* occurred in N-poor soils when supplied with rice straw[4]. Recently, Ibrahim[2] reported that organic manuring has significantly increased *Azotobacter* population and the N_2-fixation while increasing doses of nitrogenous fertilizer decreased the *Azotobacter* population in a flooded soil[1].

It is clear from the foregoing results that indigenous *Azotobacter* population could be accentuated following judicial amendments with rice straw and mineral fertilizers which would, probably, result in the high fertility status of soil under lowland rice cultivation.

The author is indebted to Prof. E. N. Mishustin, Institute of Microbiology, U.S.S.R. Academy of Sciences, Moscow.

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1. Emmimath, V. S. and Rangaswami, G., *Mysore J. agric. Sci.*, 1971, 5, 39.
2. Ibrahim, A. N., *Acta Agron. Acad. Sci., Hung.*, 1972, 21, 141.
3. Iswaran, V., *J. Ind. Soc. Soil Sci.*, 1960, 8, 107.
4. Jensen, H. L., *Proc. Linn. Soc N.S. Wales*, 1940, 61, 1.
5. Mishustin, E. N., *Plant and Soil*, 1970, 32, 545.
6. Rangaswami, G. and Subbaraja, K. T., *Proc. Ind. Acad. Sci.*, 1962, 56, 174.
7. —, and Sadasivan, K. V., *J. Ind. Soc. Soil Sci.*, 1964, 12, 43.
8. —, and Venkatesan, R., *Microorganisms in Paddy Soil.*, Annamalai University, Annamalainagar, Madras, 1966.

EFFECT OF PLANOFIX—AN NAA FORMULATION ON GROUNDNUT VAR. TMV 7

THE effect of growth regulators on groundnut yields, have been studied by Narayanan and Gopalakrishnan[1], Narasimha Rao and Gideon[2] and Wittwer and Sell[3]. Gopalakrishnan and Srinivasan[4] reported earlier significant effect of Planofix—an NAA formulation employed as foliar spray in increasing groundnut yield. Not much attention appears to have been bestowed on the effect of Planofix on nodulation and nitrogen fixation. Planofix was applied at 40 and 60 days after sowing groundnut with concentrations, 0, 5, 10, 20, 40, 80 and 160 ppm. against control (no spray). A study of the nodule number, nodule weight as also total nitrogen[5] and the total carbohydrate[6] content were made at harvest stage.

Planofix had a beneficial effect and a concentration trend both with a number and weight of nodules was quite evident (Table I). A concentration of 20 ppm was optimal for maximising nodule number while 40 ppm was optimal for maximising nodule weight. Again, 40 ppm concentration was optimal for maximising total nitrogen and carbohydrate content. With a consequent effect on maximising C/N ratio (Table II), nitrogen content of nodules increased by 31% while carbohydrate content increased by 33%. The exact role of Planofix as an NAA formulation deserves study in depth. That it has

TABLE I

Effect of Planofix on the Nodule Number and Nodule weight (mg/plant) at havrvest in Arachis hypogaea (*Var. TMV 7*)

Treatment—Planofix (ppm)	Nodule Number (per plant)	Nodule Weight (mg)
Control — No spray	57	79·60
Nil — Water spray	59	73·20
— 5	79	110·00
— 10	82	101·00
— 20	95	138·20
— 40	92	142·40
— 80	70	78·00
— 160	65	69·10

TABLE II

The effect of Planofix on the Total Nitrogen (mg/g) and the total carbohydrate (mg/g) content in Arachis hypogaea (*Var. TMV 7*)

Treatment—Planofix (ppm)	Total Nitrogen (mg/g)	Total carbo-hydrate (mg/g)	C/N ratio
Control — No spray	20·80	105·00	4·11
Nil — Water spray	23·00	110·00	4·78
— 5	25·80	120·00	4·65
— 10	26·20	129·00	4·92
— 20	26·30	132·00	5·01
— 40	27·20	140·00	5·15
— 80	26·20	125·00	4·77
— 160	25·80	80·00	3·10

its impact on nodulation and nitrogen turnover is an interesting complementary effect.

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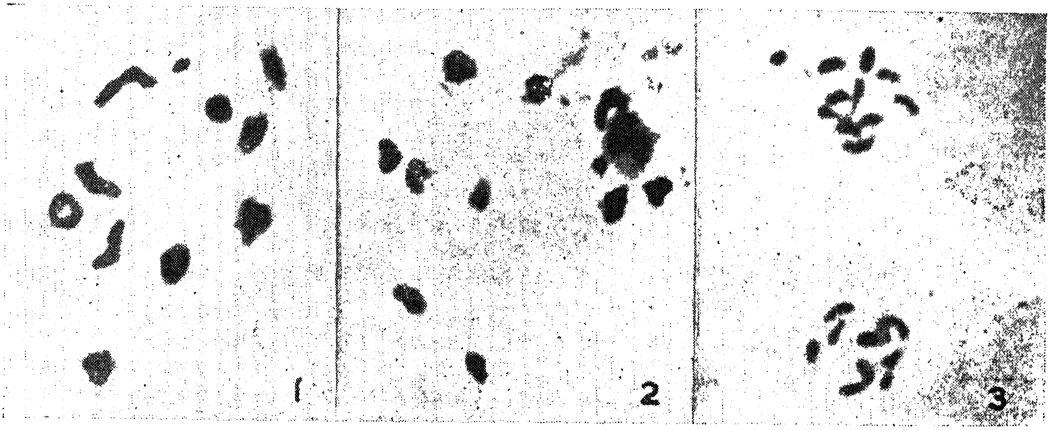
1. Narayanan, T. R. and Gopalakrishnan, S., *Modras Agric. J.*, 1952, **38**, 100.
2. Narasimha Rao, V. L. and Gideon, C. A., *Indian Oilseeds*, 1957, 1 (1), 347.
3. Wittwer, S. G. and Sell, H. M., *Effect of Growth Regulators on Plant Composition*, Mich. State Univ. Symp., 1960.
4. Gopalakrishnan, S. and Srinivasan, P. S., *Pesticides*, 1975, 9 (5), 23.
5. Humphries, E. C., *Modern Method of Plant Analysis*, Eds., Reach, K. and Tracey, M. V., 1956, 1, 468.
6. Somogyi, M., *J. Biol. Chem.*, 1952, **200**, 145.

CYTOGENETIC BEHAVIOUR OF A FRAGMENT CHROMOSOME IN JOB'S TEARS, *COIX LACRYMA-JOBI* L.

NATURALLY occurring chromosomal abnormalities in Job's tears (*Coix lacryma-jobi* L., $2n = 20$) are scarce[1]. In 1966, in selfed progenies of one of the varieties, called *typica* Watt, a single plant having quite normal appearance showed an additional fragment chromosome in tapetal and pollen mother cells. A study of chromosome associations at diakinesis and metaphase I revealed one heteromorphic trivalent and 9 bivalents, as the chromosome fragment paired with two others, in 21 (43·8%) of the 48 cells studied. In all the cells at diakinesis, the trivalent was associated with the nucleolus (Fig. 1). In cells in which 10 bivalents and one univalent were formed, since the univalent-fragment was always found attached to the nucleolus at diakinesis (Fig. 2); it is conceivable that the fragment contained the nucleolus organizing region. Types of trivalents observed were chains and frying-pan, both kinds occurring in about equal proportions. Heteromorphic bivalents involving the fragment and one member of the pair of homologous chromosomes were, however, not observed. Average chiasma frequency per cell worked out to 19·61. At metaphase-I, the fragment either showed precocious movement to the poles or was oriented on the plate along with other bivalents. At anaphase-I and telophase-I, it lagged on the spindle in 23·1% of the 52 cells studied. In 33·3% of the 33 cells examined at telophase-I, it was found staying away at one pole from the rest of the group of normal chromosomes (Fig. 3) indicating that it is frequently eliminated from the daughter nuclei. This is evidenced further from the fact that in 62 (66·0%) of the 94 pollen quartets studied it was found as a micronucleus. Pollen fertility and seed set respectively were 72·4% and 48·7% compared to 84·8% and 62·5% in the normal sibs. The plant with the fragment was maintained through vegetative propagation for several years. In the florets of the new suckers also the fragment was present indicating that it is capable of successfully carrying through mitotic cycles and, therefore, is centric. In 1968, in order to study the frequency of transmission of the fragment to the progenies, two of the suckers of this plant were selfed, three suckers were used as female and another three were used as male in crosses with the normal plants. The progenies were studied cytologically in 1969 and the results are shown in Table I.



FIGS. 1–3. Meiosis in Job's tears with $2n = 20 + 1$ fragment (× 1,000). Fig. 1. Diakinesis: 1 III and 9 II. Fig. 2. Diakinesis: 10 II and 1 I (fragment). Fig. 3. Telophase-I: 10 : 10 and the fragment at the upper pole.

TABLE I

Transmission frequency of the fragment chrmosome to the progenies under self-pollination and controlled cross-pollinations in Job's tears

Pollinations	Progeny		
	$2n = 20 + 1f$	$2n = 20$	Total
1. $2n = 20 + 1f$ selfed	2	18	20
2. $2n = 20 + 1f \times 2n = 20$	2	20	22
3. $2n = 20 \times 2n = 20 + 1f$	..	24	24

f = fragment.

Although the number of plants examined in each of the progenies of the selfed and controlled crosses was not high, it is quite apparent from the data that the fragment was transmitted through the female gametes in a low proportion of the cases (items 1 and 2, Table I), whereas its transmission *via* male gametes was not observed (item 3, Table I). Probably the male gametes carrying the fragment were either non-functional or incapable of competing with the normal male gametes in fertilization.

The origin of the fragment chromosome, however, is not known.

Our thanks are due to Mr. R. Nageswara Rao for his assistance in photographic work.

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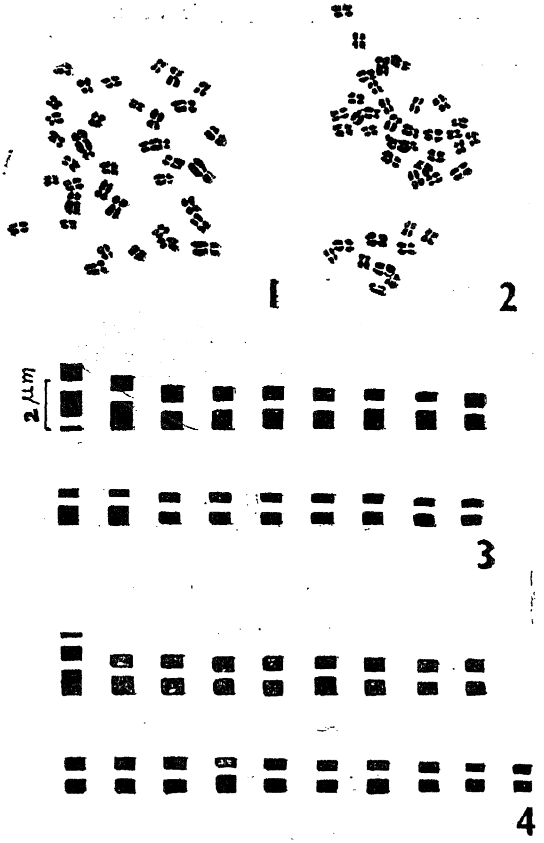
1. Venkateswarlu, J. and Chaganti, R. S. K., *Job's Tears*, Tech. Bull. No. 44, Indian Council of Agricultural Research, New Delhi, 1973.

CYTOLOGICAL STUDIES IN *FUIRENA* ROTTB. (CYPERACEAE)

THE earlier cytological information pertaining to the genus *Fuirena* concerns only the chromosome numbers of some Indian species[1-3]. The present contribution deals with the karyotype and meiosis in *F. uncinata* (Willd.) Kunth and *F. trilobites* C. B. Clarke.

Fuirena uncinata ($2n = 36$).—The length of chromosomes in the somatic complement ranges from 0·7 μm to 1·8 μm. The total length of diploid complement is 40·3 μm. The karyotype consists of 10 pairs of chromosomes with median centromeres and 8 pairs with submedian centromeres, the longest pair of the latter type possesses satellites in the long arms (Figs. 1, 3).

Fuirena trilobites ($2n = 38$).—The chromosomes in the somatic complement range from 0·7 μm to 1·7 μm in length, the total length being 40·9 μm. There are 17 pairs of chromosomes with median centromeres, and 2 pairs with submedian centromeres one of which having satellites in the short arms (Figs. 2, 4).



FIGS. 1–4. 1, 3 *Fuirena uncinata*; 2, 4. *F. trilobites*. 1, 2. Somatic chromosomes; 3, 4. Idiograms.

Meiosis is normal in both the species.

Four different chromosome numbers have been reported in the 5 Indian species of *Fuirena* that have been investigated so far. These are not in multiples of a single basic number indicating the presence of aneuploid series in the genus. *F. uncinata* with $2n = 36$ represents the lowest level of the series. While *F. ciliaris*[2,3] and *F. trilobites* show $2n = 38$, *F. wallichiana*[1] and *F. umbellata*[3] show $n = 38$ and 26 respectively. *F. wallichiana* appears to be a polyploid derived from $x = 19$. Therefore, the indication of both polyploidy and aneuploidy is evident in *Fuirena*. Besides the difference in chromosome number, the 2 species studied here reveal certain differences with respect to their karyotypes (*cf.* idiograms). These differences appear to indicate the role of structural alterations in the evolution of species in the genus *Fuirena*.

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---

1. Nijalingappa, B. H. M., *Taxon*, 1972, 21, 683.
2. —, *Ibid.*, 1975, 24, 370.
3. Rath, S. P. and Patnaik, S. N., *Bot. Mag. Tokyo*, 1974, 87, 333.

MEIOSIS IN *GRAPTOPHYLLUM PICTUM* GRIFF

THIS communication concerns the first record of chromosome number, besides giving the meiotic behaviour in *Graptophyllum pictum*, an ornamental shrub of Acanthaceae.

From an analysis of 60 pollen mother cells, 30 at diakinesis and 30 at metaphase I, the haploid chromosome number of this species is determined to be $n = 18$ (Fig. 1). Meiosis is irregular and is characterised by the presence of quadrivalents (6·6% at diakinesis and 5·9% at metaphase I) and trivalents (10·2% at diakinesis and 11·1% at metaphase I). However, their number is very low when compared to the high frequency of bivalents (60·9% at diakinesis and 60·8% at metaphase I). The multivalents are more often of the chain type with terminal chiasmata. Next to bivalents, univalents were present in high frequency (21·8% at diakinesis and 22·2% at metaphase I). Anaphase and telophase segregations were highly irregular with 1–6 laggards and micro-

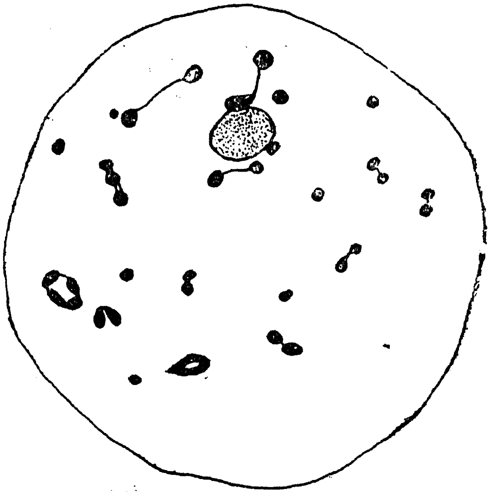

FIG. 1. Diakinesis showing $n = 18$ number with 1 IV + 1 III + 10 II + 9 Is.

nuclei respectively. In some cells triads were recorded which might have been formed as a consequence of the inhibition of the homotypic division in one of the poles. Further, six nuclei of more or less equal size were observed indicating the formation of multipolar spindles. Nearly half of the pollen grains formed are sterile (52·7%). An interesting feature observed besides polyspory is the presence of polymorphic pollen grains ranging in size from 5·0 microns to 21·5 microns (Fig. 2).

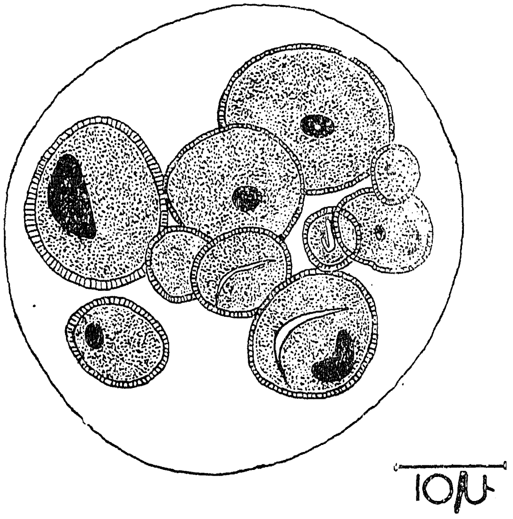

FIG. 2. Polymorphic pollen grains.

The subtribe Eujusticieae to which the genus *Graptophyllum* belongs[2] is characterised by having base numbers $x = 10, 13, 17$ and 18[1]. Since the species investigated now does not seem to be a diploid, the possibility of $x = 18$ as the base number is ruled out. Because of the absence of higher associations above the quadrivalent level, the species might be a tetraploid.

The high frequency of univalents compared to multivalents may suggest that the species is of hybrid origin. Hence this taxon may be regarded as a segmental allotetraploid, the hypothetical origin being hybridization between two diploid species ($n = 9$) followed by the doubling of the chromosome number. Polyploidy coupled with hybridization is responsible for the aforesaid meiotic irregularities which account to some extent for the high pollen sterility and lack of seed set in this species.

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---

1. Ellis, J. L., *Sci. and Cult.*, 1962, 28 (4), 191.
2. Hooker, J. D., *Flora of British India*, Vol. IV, Reeve and Co., Ltd. The Oast House Brook, Ashford, Kent, 1885.

SCREENING OF FLOWERS IN INTERSPECIFIC CROSSES OF SOME SPINOUS SOLANUM SPECIES

BY and large many previous attempts to hybridize the different spinous Solanum species have yielded either inconsistent results or have failed, and undetermined genetic factors are assumed for these results[1,2,4]. We have been reinvestigating the cytogenetics of these species and our initial findings on their flower structure, and judicious selection of them have enabled us to produce hybrids in many crosses. These are reported in this note.

The customary bagging techniques initially did not yield any positive results. The species being mostly self-pollinated, and large-scale fruit formation being prevalent in nature, self-sterility factors were ruled out. A closer examination of tagged flowers, in selected plants, showed that some of them drop off, both when left for open pollination and on hand pollination. These are usually the flowers having shorter than the normally exserted styles. Squash preparations of the ovules from these flowers revealed that the female gametophytes in them did not progress beyond the two- or four-nucleate stage of the embryosac. They are thus rendered abortive. Such an abortive development of the embryosacs was correlatable with the stylar heteromorphism[3] of the flowers. While 'normal' flowers with styles exserted well beyond the connate anthers had 8-nucleate embryosacs and were functional, the 'abnormal' ones with shorter styles were sterile as pistillate parents. The frequency with which the normal and abnormal flowers occurred on the same or different plants of any species (or between species) was variable and inconsistent. It therefore became apparent that *only* the flowers with normal exserted styles are functional as pistillate parents.

With this realisation, 483 crosses were made involving five species (8 combinations) and we have obtained 155 hybrids in all. These results suggest that selective hand pollinations confined to those pistillate flowers with normal exserted styles yield better fruit and seed set than those made on a random basis, and that stylar heteromorphism is an important factor in the reproductive potentialities of a given species.

Besides the above selective pollinations of flowers screened for their potentialities, other physical factors like the customary techniques in artificial crosses seemed to be obstacles in producing hybrids. The usual techniques involve emasculation of the pistillate parents followed by bagging the flowers before and after dusting the pollen from the desired male parent. For most of the slender pedicelled flowers of many spinous Solanums, the bags used in our initial experiments were either too small or too large, either way damaging the flowers or inhibiting their further growth. To eliminate this possible source of damage, small polythene caps (open at one end only—3 mm dia × 6 mm long) were inserted over the stigmatic heads of normal flower buds, one day prior to their opening. The next day, the closed end of the caps were cut open and pollen of the selected male parent dusted on to the stigmatic heads with a brush through the cut-open end of the polythene cap. The need for emasculation, and using the cumbersome paper-bags are thus eliminated.

The screening for the right kind of the pistillate flowers and the modified pollination technique have, in our experience, yielded better results than was otherwise possible. This procedure could be adopted to plants having small flowers with slender pedicels.

The cytogenetic aspects of the parental and hybrid genomes will be considered elsewhere.

One of us (S. V. R.) is thankful to C.S.I.R. for the award of Junior Research Fellowship.

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---

1. Bhaduri, P. N., *Indian J. Genetics and Plant Breed.*, 1951, 2, 75.
2. Magoon, M. L., Ramanujam, S. and Cooper, D. C., *Caryologia*, 1962, 15, 151.
3. Mosharraf Hossain, *Bot. J. Linn. Soc.*, 1973, 66, 291.
4. Rajasekaran, S., *Annamalai Univ. Agric. Res. Annual*, 1969, 1, 49.

CHROMOSOME PAIRING IN THE *SOLANUM SURETTENSE* × *S. MELONGENA* F_1 HETEROZYGOTE

The crossability relationships among the spinous Solanums are imperfectly understood largely due to the non-availability of viable interspecific hybrids[1,2,4]. To reevaluate the species compatabilities, we made artificial crosses of seven species ($2n = 24$), in different combinations, and have obtained variable numbers of hybrids. The chromosome behaviour at meiosis in *S. surettense* (= *S. xanthocarpum* × *S. melongena*) which differs from an earlier report on a similar F-1[2,3] are reported in this note.

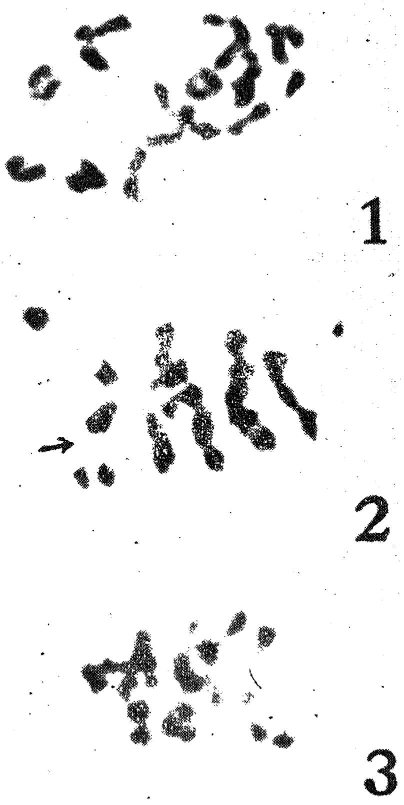
Following a selective hand pollination technique, 29 crosses were made, and 18 fruits were realised. The number of seed per fruit varied from 299 to 61 with an average of 184, 90% of which were viable. Data presented in Table I relate to stocks 1974–107–1 to 12.

TABLE I

Table showing chromosome associations and their frequencies at diakinesis and metaphase I in the F_1 *of* Solanum surettense × S. melongena ($2n = 24$)

Chromosome associations per nucleus					Number of nuclei analysed	
Ring of chain of					Diakinesis	Metaphase
V	IV	III	II	I		
1	..	..	9	1	1	..
..	3	..	6	..	1	..
..	2	..	8	..	2	3
..	1	..	10	..	17	4
..	1	..	9	2	3	..
..	1	..	7	6	1	..
..	..	1	10	1	11	1
..	..	1	9	3	4	..
..	..	..	12	..	24	5
..	..	..	11	2	17	..
..	..	..	10	4	4	..
..	..	..	9	6	1	..
..	..	..	8	8	1	..
Total number of cells analysed:					87	13

In a total of 100 pollen mother cells studied at diakinesis and metaphase I, the chromosome associations varied from nucleus to nucleus. Twelve bivalents (both ring and rod type) were seen only in 29 nuclei; in the rest, higher chromosome associations as well as univalents were observed. One to three rings or chains of four or a chain of three were seen in 31 and 16 nuclei respectively; univalents ranging from 1–8 were observed in nearly 50% of the nuclei (Table 1). Photomicrographs representing some of the chromosome associations are shown in Figs. 1, 2 and 3.



FIGS. 1–3. Fig. 1. Part of PMC at diakinesis showing a chain of four, nine bivalents and two univalents. Fig. 2. Diakinesis—Metaphase I with one ring of four and ten bivalents. Fig. 3. Metaphase I with a chain of four (extreme right) and ten bivalents (all × 1,500).

While the occurrence of 29 nuclei (at diakinesis and metaphase I) with 12 bivalents immediately suggests that the chromosomes of the two species have sufficient homologies to pair in the F_1, formation of one or more higher chromosome associations clearly shows that a given chromosome of one parent also has homologies with more than one of them in the other parent. Extensive chromosomal repattering—interchanges—is apparently the most significant cytological difference separating the two species. These results are in total disagreement with the findings of Rajasekaran[3], who observed formation of 12 bivalents in his material which was completely sterile. Some of our plants were fertile (upto 15%) and have yielded some F_2 and back-cross progenies. The reasons for this divergence in the chromosome behaviour at meiosis and the reproductive potentialities of F_1 hybrids of similar (but not identical) parentage need to be understood. Further studies on these and other aspects are in progress.

One of us (S. V. R.) is thankful to the CSIR, for the award of a Junior Research Fellowship.

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---

1. Magoon, M. L., Ramanujam, S., and Cooper, D. C., *Caryologia*, 1962, 15, 151.
2. Rajasekaran, S., *Annamalai Univ. Agric. Res. Annual*, 1969, 1, 49.
3. —, *Caryologia*, 1971, 24, 261.
4. Rao, B. G. S., *Eco. Bot.*, 1972, 26, 189.

GROWTH PATTERN IN JUTE (*CORCHORUS OLITORIUS* L.)

FOR improving fibre-yield of jute, plant selections are made on the basis of its height and base diameter. Further, a variety, which attains a large plant size within a reasonably short time, is likely to be preferred, particularly because the tenacity of fibre decreases with advancing age (Maiti *et al*, 1975).

The present investigation was undertaken at the Government Farm, Chinsurah, in 1973 with 22 types* of *C. olitorius* for studying the rate of growth in respect of plant height, base diameter and yield of fibre. The layout of the experiment was randomised blocks with six replications, each plot being represented by a row of 15 plants. Plant heights (P.H.) and base diameters (B.D.) were recorded on 5 plants selected at random from each row of one block and kept fixed, at 10-day intervals, from 80 to 130 days' crop age. The remaining five blocks were harvested one after another, at intervals of 10 days. At harvest, 10 plants were selected at random from a row and fibre was extracted and weighed. In the second set, five types were selected at random and P.H. and B.D. of ten plants of each were recorded for all the five harvests.

One series of data consisted of P.H. and B.D. values of each selected plant at different points of time and another of fibre yields of two bundles of 5 plants each of different ages of a type.

Since clear trends were not found from the fibre yield data of different sets of 5 plants, a regression of yield (y) on P.H., B.D. and age (x) was calculated from the second set of data ($y = - 3\cdot3312$

+ 0·0145 P.H. + 2·2731 B.D. + 1·2986 x), and P.H. and B.D. values of the same plants, recorded at 10-day intervals in the first set, were then fed into this regression for estimating the yields of the types at different time points.

The increments in P.H., B.D. and the estimated yield of all the types showed, on the whole, an increasing trend followed by a decreasing one. The data were fitted to a second degree curve for locating the point where the maximum occurred. In the case of yield, the logistic curve was also fitted to see the agreement between the two methods.

The 10-day periods, during which the increment was maximum in respect of all the three characters as indicated by the second degree curve, and the points at which the rate of fibre-growth was maximum according to the curve of increments (second degree) and the curve of yields (logistic), respectively, were determined. A more or less sigmoid pattern of growth was followed by P.H., B.D. and yield, particularly the first. Further, in most cases, B.D. increased at an increasing rate for a longer period that P.H., and the time points, upto which fibre grew at an increasing rate was intermediate between P.H. and B.D. According to the two curves, one of increments and another of fibre yield, the point of maximum growth rate of fibre yield of most of the types was between 95 and 105 days, the mode being near 100 days. The agreement between the values of maximum growth obtained by the two methods is quite close in a number of types. Types, 1, 3, 5, 7, 9, 10, 12, 13, 14, 15 and 22, for which the point of maximum growth is near for about 100 days can be tentatively grouped together and 6, 19 and 20 in which the point is earlier, can be included in another group. Types 2, 4, 8 and 11 can possibly be put together in an intermediate group. Those, for which values do not agree, have not been assigned to any group. There appears to be a tendency of the growth rate to fall more slowly when the peak occurs at an earlier age.

An attempt was also made to use the logistic curve of growth to determine the optimum date of harvest. It was found that if a loss in yield of 10% could be afforded, the types could be harvested at ages between 110 and 114 days, *i.e.*, a week or ten days earlier.

The authors are grateful to ICAR and the Directorate of Agriculture, W.B., for the facilities provided.

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* *Types used* : 1. Chensura Green, 2. JRO 632, 3. JRO 753, 4. Sudan Green, 5. Bangkok-I, 6. JRO 620, 7. Least pigmented, 8. Crumpled leaf, 9. Olitorius Red-I, 10. Olitorius Red-II, 11. R-26, 12. O 50-4963, 13. O 59-471, 14. Salyout, 15. Wild Olitorius Red, 16. Black Grey Seed, 17. Small Seed, 18. JRO 878, 19. JRO 4362, 20. JRO 7835, 21. Tanganyika-I, 22. Tanganyika-II.

1. Maiti, S. N., Neogi, A. K. and Sen, S., *Curr. Sci.*; 1975. 44, 274.

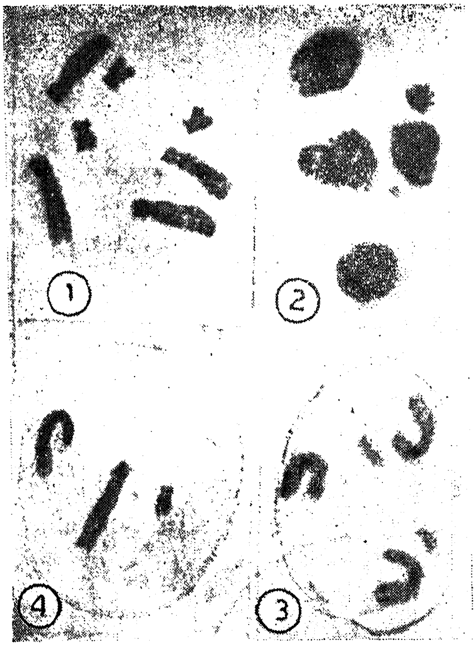
SUB-HAPLOID POLLEN IN *ALOE BARBADENSIS* MILL.

One of the several advantages of studying pollen mitosis is the opportunity it provides for characterizing haploid chromosomal complements, especially in plants showing irregular meiosis. While testing pollen fertility and studying pollen mitosis in an ecotype of *Aloe barbadensis,* the author came across a few cases of sub-haploid pollen. A preliminary report of such pollen grain is presented here. Fresh anthers were squashed in aceto-carmine and pollen with intact wall alone were considered for scoring deficiency.

Clones of *A. barbadensis* growing here are self-incompatible[1] and show as high as 60% pollen sterility. As a result, there is no fruit setting. Meiotic irregularities in this plant leading to high pollen sterility have been reported earlier by the author[2].

A majority of the functional microspores show typical haploid complement of 7 chromosomes (Fig. 1). It comprises four long and three short more or less acrocentric chromosomes typical of the tribe Aloinae. The sterile pollen grains are irregular in shape, do not take stain and are enucleate at maturity. While studying first mitosis in the microspores, a few were noted with less than seven chromosomes. Meiosis in this plant is highly irregular, resulting in bridges and laggards of various types. This leads to the formation of micronuclei and polyspory (Fig. 2). The deficient pollen originate from such abnormal microspores formed due to aberrant anaphase movements.

In higher plants, deficient microspores usually abort. They are unable to complete the mitotic divisions and the development necessary to form gametophytes. Such pollen grains are also smaller than normal and appear empty. Pollen grains even with just one chromosome less than the haploid complement fail to develop[3-6]. In the present plant, however, some of the deficient pollen are nearly normal in size, synthesize the pollen wall, but are usually less stained and do not seem to develop further into gametophytes. The chromosomes d become double indicating a normal cycle of DN synthesis. However, they do not seem to enter anaphase perhaps due to failure of spindle formation.



FIGS. 1–4. Fig. 1. A normal microspore in mitosis showing seven chromosomes in metaphase. Fig. 2. Abnormal tetrad showing micronuclei and deficient microspores. Figs. 3 and 4. Pollen grains of mitosis. All figures, × 1,650.
with $n = 5$ and $n = 3$ respectively in metaphase

Pollen grains with $n = 5$ (loss of one long and one short chromosome) and $n = 3$ (loss of two long and two short chromosomes) are found to proceed normally upto metaphase (Figs. 3 and 4). Considering the total haploid chromosome complement length in this plant[2], these pollen show dificiencies of approximately 20% and 41% respectively. Pal and Khoshoo[7,8], who have considered the chromosome number rather than chromosome length for calculating the extent of deficiency in the pollen, record about 94% deficiency in the pollen of *Amaranthus* hybrids. Some of these grains had a single chromosome instead of the usual 17, and inspite of such extreme deficiency the pollen grains were found to develop a normal wall and to enter mitosis. A clone of *Zephyranthes peurtoricensis* with $2n = 25$ has also been reported to produce pollen with $n = 1$ to 16, 20 and 24[9]. It will be interesting to culture such pollen to obtain chromosomally deficient pollen-plants.

Author is grateful to Prof. K. B. Deshpande for facilities and interest.

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1. Bailey, L. H., *The Standard Cyclopedia of Horticulture,* The Macmillan Co., N.Y., 1961.
2. Sapre, A. B., *Cytologia,* 1975, 40, 525.
3. Celarier, R. P., *Ibid.,* 1955, 20, 69.
4. Conger, A. D., *J. Hered.,* 1940, 31, 339.
5. Darlington, C. D. and Mather, K., *J. Genet.,* 1944, 46, 52.
6. Wilson, J. Y., *Cytologia,* 1959, 23, 435.
7. Pal, M. and Khoshoo, T. N., *Curr. Sci.,* 1966, 35, 614.
8. — and —, *Genetica,* 1972, 43, 119.
9. Raina, S. N. and Khoshoo, T. N., *Ibid.,* 1971, 42, 239.

PROTEOLYTIC ENZYME CHANGES IN MANGO SHOOTS AS AFFECTED BY GROWTH RETARDANTS IN RELATION TO FLOWERING*

THOUGH it has been proved recently that flowering could be induced in mango during an 'off' year by applying growth retardants such as cycocel (2–chloroethyl trimethyl-ammonium chloride) and Alar (N, N-dimethylamino succinamic acid)[2,3], the physiological and biochemical changes that take place following the application of such potent chemicals is not known in mango. Hence, among other aspects, the pattern of seasonal changes of proteolytic enzyme as influenced by growth retardants was studied in 'Mulgoa,' an irregular cultivar of mango. Cycocel and Alar were applied as aqueous sprays at 5000 ppm concentration at monthly intervals starting from May (eighty sprays, upto December), on selected branches of two healthy trees. Leaves of treated shoots were used for the estimation of proteolytic enzyme activity at monthly intervals, one week after spraying. The enzyme activity of leaf extracts was measured based on the aromatic amino acids released by native enzyme from a standard solution of casein[1].

The time of flower bud formation was November in spring flush shoots. Both Alar and cycocel caused an appreciable increase in the percentage of flowering shoots (Table I). A relation between the amino acid increases and a concomitant decrease in protein content of shoots, and *vice versa,* have been observed in the present studies. The enzyme activity gradually increased in the leaf throughout the period of observation, and the retardants significantly reduced this activity (Table III), which must have obviously been responsible for retardation of protein degradation under the treatments. It can therefore be assumed that the growth retardants have a beneficial effect on protein metabolism by regulating its metabolic processes. It is concluded that, by this regulative effect, the growth retardants cause the maintanance of a high level of proteins (Table II) in the shoots perhaps ultimately leading to higher percentage of flowering in the treated shoots (Table I).

TABLE I

Extent of lowering as influencing by growth retardants

Year	Percentage of flowering shoots		
	Control	Cycocel	Alar
1972–73	24·75	84·75	46·75
1973–74	66·00	83·25	71·25

TABLE II

Changes in proteins as influenced by growth retardants (*Per cent on dry weight basis*)

Month	Control	Cycocel	Alar
September 1973	4·85	5·12	5·12
October 1973	4·81	4·96	4·92
November 1973	3·99	4·67	4·57
December 1973	4·02	4·44	4·25
January 1974	4·07	4·23	4·12

S.E.		*C.D.* ($P = 0·05$)
(i) Treatments	0·0121	0·034**
(ii) Months	0·0210	0·060**
(iii) Treatments × months	0·0514	0·149**

TABLE III

Proteolytic enzyme activity (*in trytophan units*) *in leaf during* 1973–74 (*var. Mulgoa*)

Month	Control	Cycocel	Alar
September 1973	0·75	0·65	0·62
October 1973	0·93	0·75	0·72
November 1973	1·04	0·88	0·81
December 1973	1·60	1·37	1·25
January 1974	1·82	1·54	1·44

S.E.		*C.D.* ($P = 0·05$)
(i) Treatments	0·0031	0·010**
(ii) Months	0·0041	0·012**
(iii) Treatment × months	0·0071	0·021**

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1. Davies, N. C. and Smith, E. L., *Methods Biochem. Anal.*, 1955, 2, 215.
2. Maiti, S. C., Mukhopadyaya, A. K. and Sen, P. K., *Curr. Sci.*, 1971, 40, 388.
3. —, Basu, R. N. and Sen, P. K., *Acta Horticulturae*, 1972, 24, 192.

PALYNOLOGICAL EVIDENCE ON THE AGE OF MIKIR FORMATION

NON-marine sediments of North Cachar Hills known as the Mikir Formation, unconformably overlie th denuded surface of the Archaean Complex. Thi formation is overlain by the marine Garampani Limestone. A rich palynological assemblage has bee recovered for the first time from this stratal sequenc The assemblage mainly consists of pteridophyt spores with angiospermic pollen in subordinate amoun and compares closely with the palynological assemblages of Tura and Cherra formations of Shillon Plateau. This favours a Palaeocene-Lower Eocen age for the Mikir Formation instead of a Cretaceous dating as proposed by the earlier workers.

The name Mikir Formation has been given b Samanta[12] (1971) to a group of sandstones, shale argillaceous limestones and siltstones with strings c coal, developed in Garampani (25° 30′ 40″ : 92° 38′ 00 area, North Cachar Hills, Assam. The formatio rests unconformably over the Precambrian compl and is conformably overlain by Garampani Limesto Medlicott[5] (1869), Latouche[4] (1883), Medlicott an Blanford[6] (1893) and Smith[13] (1893) all have designate the sedimentary strata perhaps belonging to this formation (Garampani Limestone in sense of Samanta, 1971 occurring below the nummulities in Mikir and Nor Cachar Hills as Cretaceous. Evans[2] (1932) for th first time expressed a doubt about their Cretaceou dating and favoured an Eocene age. Gangopadhya (1970) in connection with the Kopili Hydel Proje mapped the area round Garampani. He designat the lower arenaceous facies as Cherra Stage and t succeeding limestones and shales as Sylhet Limestone stage and Kopili Stage, respectively.

A large number of palynological rock samples were collected from Mikir Formation in field Seasons 1971–75 to determine its age and stratigraphical position in the light of palynological studies. Laboratory investigations have shown that the palynofossils recovered from this formation are dominated by the pteridophytes. Angiosperms are next in number. Algae and fungi are scantly represented. Stratigraphically significant taxa such as *Lycopodiumsporites palaeocenicus*, *Lygodiumsporites eocenicus*, *Cyathidites australis*, *Dandotiaspora telonata*, *D. dilata*, *Todisporites major*, *Polypodiaceaesporites tertiarus*, *Polypodiisporites oligocenicus*, *Proxapertites crassimurus*, *P. assamicus*, *Assamialetes emendatus*, *Palmidites plicatus*, *P. maximus*, *Nymphaeacidites sphericus* and *Polycolpites cooksonii* which form dominant assemblage in Cherra and Tura Formations of the Shillong Plateau, also abound in this formation.

Mikir-mioflora compares closely with that of Tura Formation of Garo Hills. *Assamialetes emendatus*, *Dandotiaspora telonata*, *Palmidites plicatus* and *Proxapertites assamicus* Cenozones instituted by Sah and Singh[10] (1974) in Tura Formation of Garo Hills are well distinguishable in Mikr Formation also. Thus the two formations seem to be synchronous. This contention is also supported by Samanta (1971, p. 325) after comparing his three foraminiferal zones of Garampani area with those of Garo Hills (Samanta,[11] 1968). Since Mikir Formation displays a close miofloral resemblance with Tura and Cherra formations of the Shillong Plateau it may also represent a Palaeocene-Lower Eocene age. Therefore, its cretaceous dating is ruled out. Predominance of continental fresh water elements and absence of any distinctive marine or brackish water form in the Mikir-mioflora further suggests the deposition of this lithounit, probably under freshwater condition.

Authors are grateful to the authorities of Birbal Sahni Institute of Palaeobotany, Lucknow, for providing facilities to carry out the present investigation.

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1. Dutta, S. K. and Sah, S. C. D., *Palaeontographica*, 1970, **131** **(B)** 1.
2. Evans, P., *Trans Min. Geol. Inst. Ind.*, 1932, **27** 155.
3. Gangopadhyay, S., *Indian Jour. Power River Valley Devl. Spl. No.*, 1970, p. 32.
4. Latouche, *Rec. Geol. Surv. India*, 1883, **16** (4), 202.
5. Medlicott, H. B., *Mem. Geol. Surv. India*, 1869, **7**, 159.
6. — and Blanford, W. T., *Manual of Geology of India*, 1893, p. 301.
7. Sah, S. C. D. and Dutta, S. K., *Palaeobotanist*, 1966, **15** (1), 76.
8. — and —, *Ibid.*, 1968. **16** (2), 127.
9. — and —, *Ibid.*, 1974, **21** (1), 42.
10. — and Singh, R. Y., *Spl. Publ. B., Sahni Inst. Palaeobotan.*, 1974, 3, 76.
11. Samanta, B. K., *Geol. Mag.*, 1968, **105** (2), 124.
12. —, *Jour. Geol. Soc. India*, 1971, **12** (4), 318.
13. Smith, F. H., *Mem. Geol. Surv. India*, 1898, **28** (1), 71.

THE PRESENT SITUATION OF PHYSIOLOGIC RACES OF *PYRICULARIA ORYZAE* CAV. IN INDIA

PADMANABHAN[1] reported the occurrence of physiologic races of *Pyricularia oryzae* Cav. for the first time from India. Padmanabhan *et al.*[2] reported the occurrence of thirty-one races of the pathogen after studying the pathogenicity patterns of one hundred and thirty-two isolates of *P. oryzae*, obtained through isolation from blast specimens collected from different States of India. One more race of the pathogen was reported to occur in India from Central Rice Research Institute[3]. Veeraraghavan and Premalatha Dath[4,5] have reported the occurrence of the physiologic races of the pathogen which could be detected by them during 1971–74. The present contribution relates to the identification of physiologic races of *P. oryzae* prevalent during 1975 in different parts of this country.

Sixty-five isolates of *P. oryzae* were obtained through isolation from blast specimens belonging to Kerala, Karnataka, Tamil Nadu, Andhra Pradesh, Orissa, West Bengal, Manipur, Himachal Pradesh, Haryana, Sikkim, Gujarat and Maharashtra. The method of raising the plants of the international blast differentials, inoculating and scoring them were essentially the same as described by Padmanabhan *et al.*[6]r. Classification of host-reaction for identification of physiologic races of *P. oryzae* suggested by Veeraraghavan and Premalatha Dath[4] was followed. Under this method 'C' spots—circular spots about 2–3 mm in diameter with central ashy zone and dark purplish-brown margin—were considered as susceptible reaction of the host as also 'D' and 'E' type of spots both of which revealed central ashy zone, brown margin and sporulation. These features are common to 'C' type of spots also. The nomenclature and classification for physiologic races of *P. oryzae* presented by Veeraraghavan[7] was adopted.

The results of the analysis revealed that all the isolates of *P. oryzae* screened in this study belonged to the race 0–17 = (IC 17 Ling and Ou). The pathogenicity pattern of this race of the pathogen is given in Table I.

TABLE I

Pathogenicity pattern of the race 0–17 of P. oryzae *on international blast differentials*

Raminad Str. 3	Zenith	N.P. 125	Usen	Dular	Kanto-51	CI. 8970 (S)	Caloro
R	R	S	R	S	S	S	S

R = resistant, S = susceptible.

Veeraraghavan and Premalatha Dath[5] could detect the presence of the physiologic races IC 1 and IC 17 as per Ling and Ou classification[8] during 1971. In the system adopted in this contribution, they will be equivalent to 0–1 and 0–17 respectively. All the one hundred and eighty-one isolates of *P. oryzae* collected by them[5,6] during 1972–74 revealed the exclusive prevalence of the race IC–17 (Ling and Ou) = 0–17 (Veeraraghavan). The current contribution reveals the continuation of the same trend.

The authors are indebted to Dr. S. Y. Padmanabhan, Director, for his keen interest and constant encouragement during the course of this investigation. The authors thank the scientists of the Department of Agriculture of the States of India and the universities for their kind co-operation in providing the blas specimens and to Sri. N. K. Chyau Patnaik for help in maintaining the purity of the differentials.

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---

1. Padmanabhan, S. Y., *Curr. Sci.*, 1965, **34** (10, 307.
2. —, Chakrabarti, N. K., Mathur, S. C. and Veeraraghavan, J., *Phytopathology*, 1970, **60** (11, 1574.
3. *Annual Report, Central Rice Research Institut Cuttack, India*, 1969, p. 109.
4. Veeraraghavan, J. and Premalatha Dath, A *Curr. Sci.*, 1975, **44**, 1[illegible].
5. — and —, *Ibid.*, 1976, **45**, 333.
6. Padmanabhan, S. Y., Chakrabarti, N. K., Mathu S. C. and Veeraraghavan, J., *Proc. Ind. Ac Sci.*, 1967, **66 B**, 63.
7. Veeraraghavan, J., *International Rice Commissi Newsletter*, 1975, **24** (2), 128.
8. Ling, K. C. and Ou. S. H., *Phytopathology*, 19 **59**, 339.

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ULTRACENTRIFUGAL STABILITY OF NONAQUEOUS EMULSIONS

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IT has been shown earlier[1-3] that in addition to normal O/W and W/O emulsions, it is possible to prepare a new third type of oil-in-oil emulsion (nonaqueous system) with two nonaqueous immiscible liquids as internal and external phases and surfactant as emulsifier.

Breaking of emulsions is generally considered to take place in two steps[4]: flocculation in which droplets of the dispersed phase form aggregates (this step is reversible) and coalescence which results in the ultimate separation of dispersed droplets as a bulk phase. Ultracentrifugation[5] is found to offer a means for accomplishing the flocculation step very rapidly and thus producing a highly concentrated emulsion before appreciable coalescence can occur. The coalescence process then could be studied independently. In this paper emulsion stability is used exclusively to refer to the rate of coalescence.

In the present communication the stability of nonaqueous emulsions prepared with benzene and ethylene glycol as two nonaqueous phases and sodium dioctylsulphosuccinate as emulsifier has been determined quantitatively employing an ultracentrifuge at 25,980 r.p.m.

A series of nonaqueous emulsions of benzene (BDH) in ethylene glycol (BDH) was prepared with various concentrations of emulsifier. A 1 : 1 phase ratio by volume was used and the concentration of sodium dioctylsulphosuccinate (BDH) was varied from 1 to 5% (w/v % of ethylene glycol). The heterogeneous mixtures of benzene and ethylene glycol containing anionic surfactant were emulsified with the help of Braun emulsator for final making of the O/O emulsions.

A Beckman Spinco Model E analytical ultracentrifuge was used in this work with a 12 mm light path and a 4 degree sector angle standard cell having a capacity of about 0·8 ml. Immediately after preparation of an oil-in-oil emulsion, 0·7 ml of the emulsion was injected into the ultracentrifuge cell through a hypodermic needle. The cell was then placed in an analytical rotor and subsequently cenrtifuged in the ultracentrifuge at 25,980 r.p.m. The time required for acceleration to this constant speed was 2 min and 45 sec ± 15 sec. Zero time in the experiments were taken as the time at which constant speed was reached. All runs were made at 30° ± 0·1° C.

During centrifugation three layers develop in the cell: transparent layer of benzene, the remaining opaque, concentrated stable emulsion and the transparent layer of ethylene glycol. Photographic records were made of the cell at regular time intervals during the experiment. The layers give sharp boundaries on a photographic plate which change position with time as more benzene is separated from the emulsion. Determination of the positions of the boundaries on a series of such plates taken at successive time intervals permits calculation of the rate of separation of benzene from the emulsion. Measurements were made with a travelling microscope determining the distance from the top of the cell to the benzene-emulsion and emulsion-ethylene glycol interfaces, and to the bottom of the cell.

The volume, V, corresponding to a length, *l*, in the 4° sector angle standard cell is given by

$$V = \frac{4}{360}\pi t\,[(d+l)^2 - d^2]$$

$$= \frac{\pi t l}{90}(2d+l)$$

where *d* is the distance from the centre of rotation to the top of the cell and *t* is the thickness of the cell. A graph was plotted of the volume as a function of the measured value of *l* from the top of the cell using an optical enlargement of 2·03. The plot was then used to determine the volume from the differences between the measured distances to the different interfaces.

The per cent of the initially emulsified benzene which has separated at any given time is determined by dividing the volume of the benzene layer by the sum of the volumes of the benzene layer and the creamed emulsion. Figure 1 is the characteristic of the curves

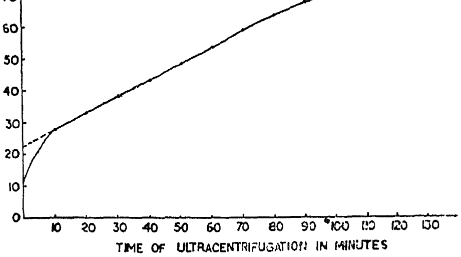

FIG. 1. Rate of separation of benzene from a 50% v benzene — 50% v ethylene glycol — 1·0% w sodium dicotyl-sulphosuccinate nonaqueous emulsion.

of per cent benzene separated *vs.* time of ultracentrifugation. In this case the nonaqueous emulsion of 50% *v* benzene in 50% *v* ethelene glycoi is stabilized with 1% *w* sodium dioctylsulphosuccinate. The rate of separation at the known speed is determined from the slope of the linear portion of the curve. The slope of the line, *i.e.*, the rate of separation of benzene in per cent per min, is termed the ultracentrifugal stability and its extrapolated intercept on the axis at zero time is the per cent benzene separated at zero time.

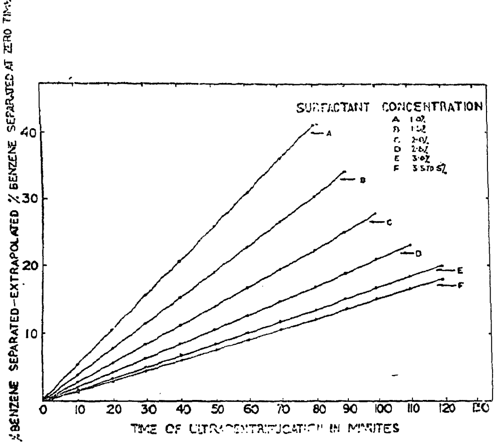

FIG. 2. Effect of initial surfactant concentration on the rate of separation of benzene from nonaqueous emulsions centrifugd at 25,980 r.p.m.

Figure 2 shows the effect of concentration of emulsifier on the rate of separation of benzene from the emulsions in the ultracentrifuge. Increasing the concentration of surfactant at first decreases the rate of separation of benzene from the emulsion and also the amount of benzene which is separated rapidly on initial centrifugation before the steady state rate is reached. At higher concentrations the rate becomes independent of the initial concentration of emulsifier, as is strikingly shown in Fig. 2, all emulsions prepared with surfactant concentration between 3·5 and 5% separating benzene at the same rate.

The results of the ultracentrifugal experiments indicate that the rate of coalescence of non aqueous emulsion droplets decreases with an increase in initial surfactant concentration until an optimum surfactant concentration is reached and thereafter is independent of concentration.

During ultracentrifugal studies, the benzene droplets in oil-in-oil emulsions have been forced by centrifugation into very closely packed arrays which can be described as aggregated emulsions. The resistance of the aggregated droplets to coalescence arises from the existence of emulsifier adsorbed on the particle surfaces. No change observed in stability of nonaqueous emulsions to centrifugation when the emulsifier concentration exceeds an optimum value, is believed to be associated with the density of packing of adsorbed emulsifier on the particle surface. As the emulsifier concentration is raised, the amount of emulsifier adsorbed on the emulsion particle increases and the aret of the particle surface per adsorbed emulsifier molecule decreases. Thus the amount of adsorption at the interface increases, with resultant decrease in the rate of separation of benzene from the emulsion. At the optimum concentration of the emulsifier, the density of packing is at its highest value with the area per adsorbed emulsifier molecule approaching the actual molecular cross section. At this point, the mechanical stability of the adsobed emulsifier layer can reasonably be expected to be greatest. The addition of emulsifier in excess of the optimum concentration should only increase the number of molecules in the ethylene glycol phase without appreciable effect on the density of packing of the adsorbed emulsifier and consequently beyond this point the ultracentrifugal stability should no longer be dependent on the concentration of surfactant.

1. Sharma, M. K., *J. Colloid Interface Sci.*, 1975, 53, 340.
2. —, *Curr. Sci.*, 1975, 44, 770.
3. —, *Kolloid-Z., Z. Polymere* (in press).
4. Becher, P., *Emulsions: Theory and Practice*, Reinhold, 2nd Ed., New York, 1965, p. 170.
5. Garrett, E. R., *J. Pharm. Sci.*, 1962, 51, 35.

EFFECT OF CERTAIN ALKYLATING CHEMOSTERILANTS ON THE OVARIAN STRUCTURE AMERICAN COCKROACH (ORTHOPTERA: BLATTIDAE)

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IN the course of an investigation on the effect of apholate on corpora allata of *Periplaneta americana* (Bhargava and Tandon)[1], it was observed hat corpora allata show clear signs of degeneration after the treatment. Since terminal oocytes in the varies of such cockroaches showed complete inhibi ion of growth and indication of resorption, it eemed of interest to see if the inhibition of oocyte levelopment and resorption is due to inhibition of ormone release from corpora allata. The following xperiment was, therefore, performed. Apholate, etepa and thiotepa were selected for the trials.

The cockroaches were bred in the laboratory on ried and powdered bread. Food of the experi ental insects was saturated with 2% solution n redistilled water) of one of the chemicals selected nd dried. The ovaries were removed, fixed in ouin's and sections were cut. Sections were ained with haematoxylin and eosin. Sections of eated cockroaches were compared with similar eparations from untreated ones (Fig. 1).

RESULTS WITH APHOLATE

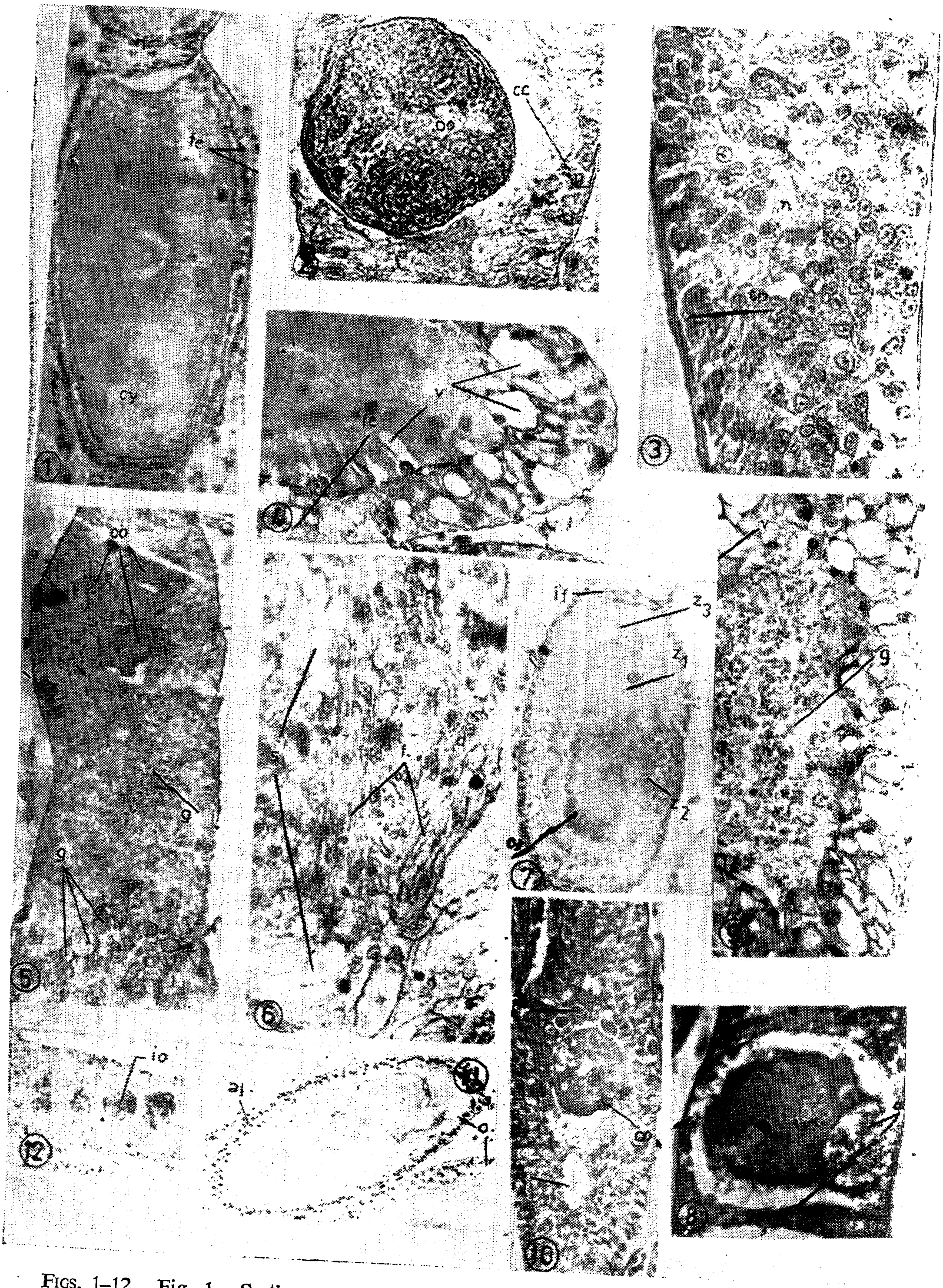
Within 24 hr a few small vacuoles appear in e peripheral cytoplasm of the mature oocytes d the follicular epithelial cells. The immature cytes start shrinking away from the follicular ithelium (Fig. 2) and the interfollicular tissue comes thin. After 72 hr, the interfollicular sue degenerates completely and thus all the llicles of an ovariole become continuous (Fig. 4). e vacuoles increase in number as well as in size. e nuclei show clumped chromatin. The presence a few small granules in the cytoplasm of the ture oocytes indicates the beginning of granular generation. After 120 hr the ovarioles start inking. This disturbs the linear arrangement of oocytes which are now seen placed side by e at the distal end of the ovariole (Fig. 5). The ernal ovariole sheath does not shrink as rapidly l gets separated from the ovariole, and, therefore, be seen projecting beyond the apex of the riole. The granules in the mature oocytes become ger and irregular in shape. After 144 hr e is notable reduction in the length and width the ovariole. This makes the elastic tunica propria thick due to contraction. By 216 hr, the immature oocytes become completely degenerated leaving only the follicular tissue in the distal end of the ovariole. There is further reduction in the size and the intensity of staining. Proximally, the ovarioles show numerous irregular solid particles out of which some stain deeper (Fig. 5). A few ovarioles show fibres at their proximal ends indicating the beginning of fibrous degeneration (Fig. 6). Prolonged treatment further reduces the size of the ovarioles, causes the discharge of solid particles leaving empty spaces (Fig. 6) and increases the vacuolization and nuclei concentration.

RESULTS WITH METEPA

The effects of metepa also become distinctly visible within 24 hr. There is a general thickening of the follicular epithelium. However, the epithelium enveloping the mature oocytes becomes more thick and shows more or less three distinct zones—the outer showing vacuoles, the middle losing the staining intensity while the inner zone remains normal. After 48 hr, the number and the size of the vacuoles in the oocyte cytoplasm increase and the germinal vesicle contents become granular. The oocyte which starts shrinking away from the follicular epithelium shows distinct zones in its cytoplasm, the inner zone showing two or more lobes of dark staining clumped chromatin material. Within 72 hr of treatment the interfollicular cells first become flat and then degenerate making the follicles continuous. The nuclei show karyorrhexis. The mature oocytes show a complete picture of vacuolar and granular degeneration (Fig. 9). After 96 hr the ovarioles show only remnants of oocytes having round or ovoid granules in the cytoplasm enveloped in thick epithelium (Fig. 10). Prolonged treatment further reduces the width of the ovarioles.

RESULTS WITH THIOTEPA

The effects of thiotepa are more or less similar to those produced by metepa (Figs. 11 and 12). However, the interfollicular tissue degenerates within 48 hr and granular degeneration sets in within



FIGS. 1–12. Fig. 1. Section of normal ovary, 10 × 10. Fig. 2. 72 hr (alpholate treated) —showing reduced cytoplasm in follicular cells and shrinkage of the oocyte, 10 × 40. Fig. 3. 145 hr.

(apholate treated)—Distal end of the ovariole showing follicular epthelium of two sides coming closer because of reduced size of oocyte. Note the presence of vacuoles and cellular degeneration in follicular tissue and thick tunica propria, 10 × 40. Fig. 4. 72 hr (apholate treated) follicles have become continuous due to degeneration of interfollicular tissue, 10 × 40. Fig. 5. 216 hr. (apholate treated)—showing distal end of ovariole with three clumped oocytes two of them placed side by side. Note the aggregation of nuclei in the centre. The proximal end of ovariole shows dark stained granules of large size, 10 × 10. Fig. 6. 216 hr (apholate treated)— proximal end of the ovariole showing fibrous degeneration. Note that the granules have been discharged leaving empty speces, 10 × 40. Fig. 7. 24 hr. (metepa treated)—The cytoplasm of the oocyte showing three different zones. Also note the vacuoles in cytoplasm. 10 × 10. Fig. 8. 48 hr (metepa treated)—Oocyte showing the bursting of the germinal visicle. Note the granulation of the material of germinal vesicle, 10 × 40. Fig. 9. 72 hr (metepa treated)—Mature oocyte showing increased thickness of the follicular epithelium and large vacuoles. Note also the granulation of cytoplasm, 10 × 40. Fig. 10. 96 hr (metepa treated)—Note the enlarged follicular tissue and only remnants of cytoplasm and granules, 10 × 40. Fig. 11. 24 hr (thiotepa treated)—Oocyte . cytoplasm showing three distinct zones. The follicular epithelium has thickened, 10 × 40. Fig. 12. (Thiotepa treated) —showing continuation of the follicles. Note clumping of the chromatin, 10 × 10.

cc—Clumped chromatin; *cv*—Cytoplasm; *f*—Fibres; *fe*—Follicular epithelium; *g*—Granules; *gv*—Germinal vesicle; *if*—Interfollicular tissue; *io*—Immature oocyte; *n*—Nuclei; *o*—Ovariole; *oo*—Oocyte; *s*—Space; *tp*—Tunica propria; *v*—Vacuoles; *z-1* to *z-3*—Zones of damaged oocyte.

72 hr. The size of the ovariole remains uneffected. Prolonged treatment produces fibrous degeneration.

Morgan[2] expressed his doubt that the affected cells may be able to overcome the effect of chemosterilants. The formation of fibrous tissue after the treatment with apholate, metepa and thiotepa clearly indicates that the damage is a permanent one. Though the nature of the damage in the ovarioles caused by all the three chemicals is more or less same, it is interesting to note that apholate affects the length and width both (as was also reported by Burden and Smittle[3]) metepa only the width while thiotepa does not affect the size of the ovariole at all.

La Brecque and Smith[4] raised a question whether chemosterilants affect fecundity and oviposition through inhibition of neuroendocrine system in insects. Bhargava and Tandon[1] report that apholate causes atrophy in corpora allata and thus inhibits hormone release in *P. americana*. Apholate has been known to affect fecundity and oviposition in many insects[5-7]. The present investigation clearly shows that oocytes are damaged beyond repair after treatment with apholate, metepa and thiotepa. As the control of oocyte maturation by corpora allata is well known[8], it is reasonable to assume that apholate damages the oocytes *via* corpora allata in *P. americana*. As the two other alkylating agents are showing almost similar effects on the oocytes, the mode of action of all the three compounds might be the same.

Thanks are due to United States Agriculture Department, USA, for generous gift of chemicals.

1. Bhargava, S. and Tandon, G. N., *Entomol. Monthly Mag.*, 1975, 111, 89.
2. Morgan, P. B., *Ann. Entomol. Soc. Am.*, 1967, 60 (4), 812.
3. Burden, G. S. and Smittle, B. J., *Florida Entomologist*, 1963, 46, 229.
4. La Brecque, G. C. and Smith, C. N., *Principles of Insect Chemosterilization*, 1968.
5. Morgan, P. B. and La Brecque, G. C., *J. Econ. Entomol.*, 1962, 55, 628.
6. Chamberlain, W. F. and Barrett, G. C., *Nature*, Lond., 1968, 218, 471.
7. Murry, W. S. and Bickely, W. E., *Marryland Uni. Exp. St. Bull.*, 1964, 134 A, 37.
8. Wigglesworth, V. B., *Principles of Insect Physiology*, 1972.

DARSHAK SEA MOUNT IN THE NORTH-EASTERN ARABIAN SEA

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ABSTRACT

The Darshak Sea Mount is situated just south of the Indus Canyon. It rises upwards from a depth of about 1,500 m to approximately 450 m towards the surface. Similar submarine hills and sea mounts are seen in the profiles and physiographic maps of that region. These sea mounts and submarine hills possibly mark the site of earlier volcanic activity probably related to Deccan Traps.

THE topography of the sea floor to a large extent reflects its geology and structure. Geomorphological maps based on the analyses of echograms thus provide useful basic information on the geology and structure of the sea bed. As a part of the Institute's project on regional geology of the

Western Continental Margin of India, a geomorphological map of this region is being compiled. This is based on the analyses of echograms of this area collected by the various ships in recent ye including some of the survey vessels.

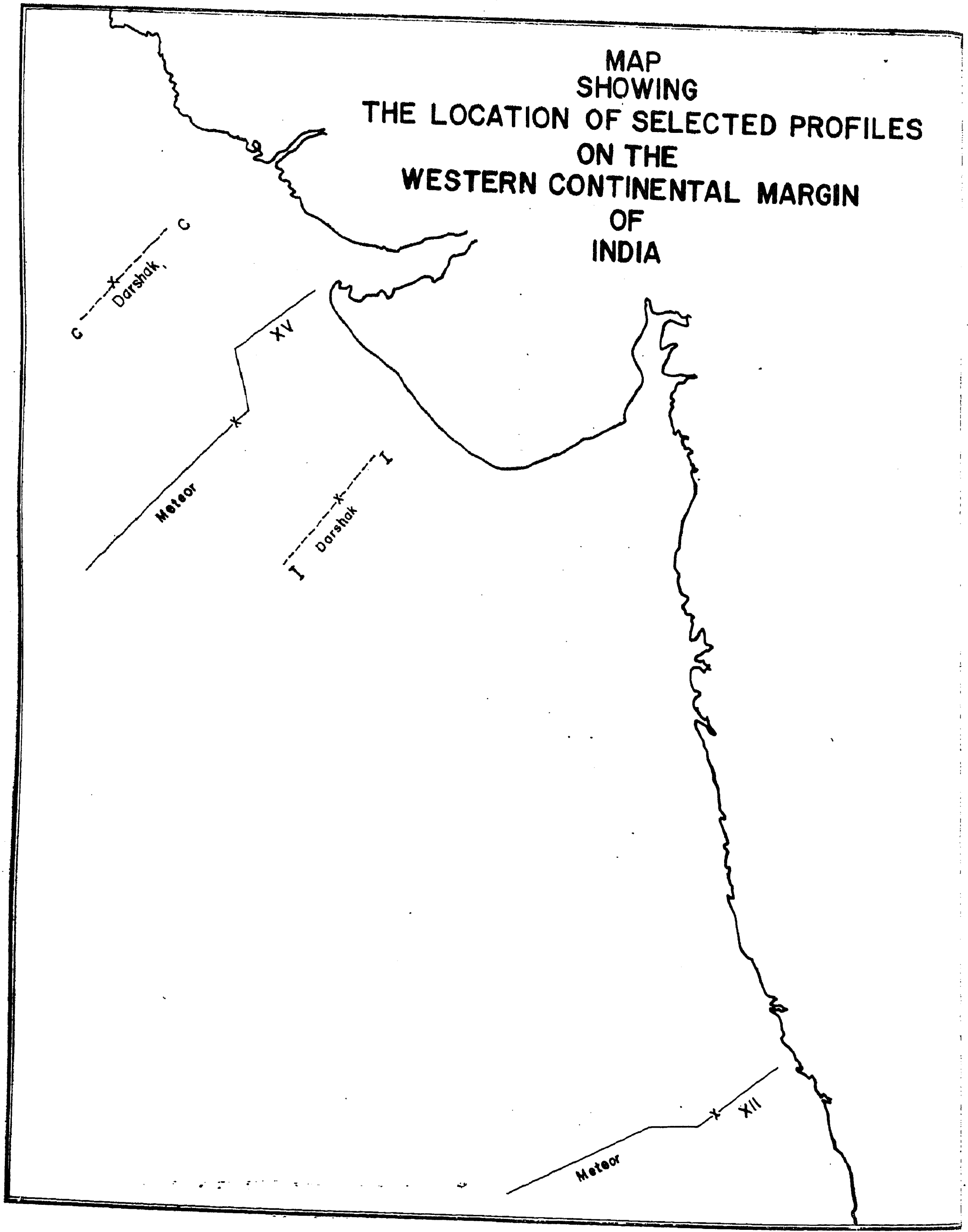

FIG.

The echograms are first used to prepare profiles showing the submarine topography. These are prepared on a horizontal scale of 1 cm to 2 nautical miles and vertical scale of 1 cm = 100 m which gives a vertical exaggeration of 1 : 37. The profiles thus prepared are used for compiling geomorphological maps. An interesting feature of some of the profiles recently prepared is the occurrence of well-defined submarine hills on the continental slope and continental rise (Fig. 1). The northernmost of these submarine hills is situated (22° 28′ : 66° 42′) just south of the Indus Canyon on the continental rise. In Track C a submarine hill rises upwards from a depth of about 1,500 metres to 450 metres towards the surface (Fig. 2). The hill has a base of about 18·5 km which is roughly reduced to 8 km at the top. The landward side has a slope of about 14° and the seaward side about 20°. Though the area has not been surveyed in detail in the recent cruise but a comparison of the existing profile with the soundings made by earlier ships indicates that the topographic high is an isolated feature within the Indus Cone sediments.

The tracks both on the north and south indicate (GEBCO Chart C 132) that the feature does not have a wide extent either towards or along the shelf. The slopes are more or less similar to those recorded on some of the Pacific sea mounts (Emery, Tracey and Ladd[2] quoted in Fairbridge, 1966). The submarine hill has a sharp peak and the topography on the top is very much rugged. These features agree well with the definition of the sea mount given by the International Committee on Ocean Bottom Features (1952), which identifies the sea

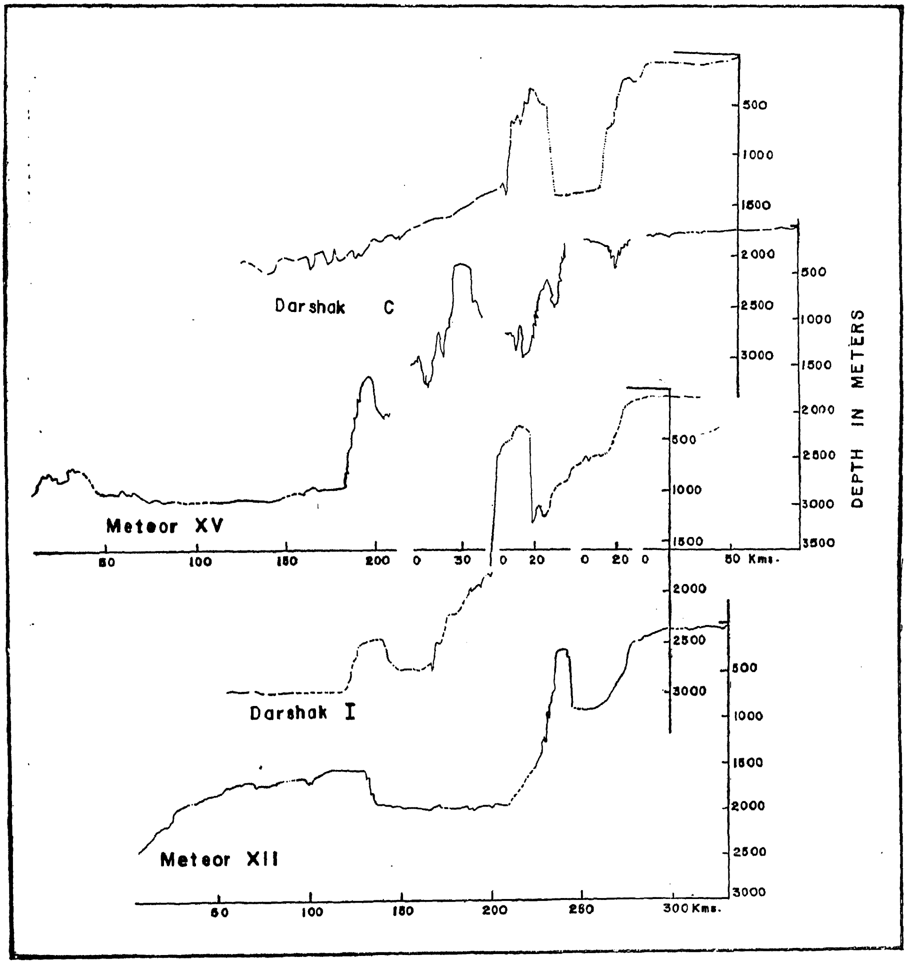

FIG. 2

mount as an isolated or comparatively isolated elevation of the deep sea floor from approximately 1,000 metres or more.

The present topographic feature is named as "DARSHAK SEA MOUNT" after the Indian Naval Ship "DARSHAK" from whose echograms it was confirmed. The seaward side of the Profile across this hill shows a characteristic depression which may be similar to the peripheral depression recorded on some of the sea mounts in the Pacific (Fairbridge, 1966)[3]. In this area the depression may also be due to its little accumulation of sediments from the Indus on the seaward side. However, more detailed studies of the sea mount are needed as the origin of this depression may be far much more complex than that of a simple model of upstream deposition (Roberts, Hogg, Bishop and Flewellen, 1974)[7]. Though the present height of the submarine hill above the sea floor is about 1,000 meters, there can be little doubt that the original relief must have been much greater as the surrounding area has been filled subsequently by the Indus sediments whose thickness in the area exceeds 2·5 km (Neprochnov, 1961)[6]. Over 2,000 sea mounts have been discovered in all major ocean basins and the recent expeditions in the Indian ocean have also reported many. The physiographic diagram published by Heezen and Tharp (1964)[4] shows a number of sea mounts in the Indian Ocean. In the north-eastern Arabian Sea, submarine hills are shown on the extension of the Laccadive Ridge and along the edge of the western continental slope of India. The other submarine hill represented in the profile (I in Fig. 2) including the present sea mount appear to be the northernmost extension of these submarine hills and may possibly represent an extension of the Laccadive trend along the western continental slope of India.

Similar submarine hills are also seen in the profiles based on the echograms (XV and XII in Figs. 1 and 2) of *RV Meteor* as far south as 24° and 14° latitude. These submarine hills and their extensions which may include the Darshak Sea Mount possibly represent a widespread occurrence of similar topography along the continental margin. Their occurrence along the western margin is of significance as it is well known that most of the submarine hills and sea mounts are submarine volcanoes (Menard, 1964[5]; Uchupi, Phillips and Prada, 1970[9]) and they are also related to fracture zones. Thus the submarine hills of the continental slope and rise may possibly represent a period of extensive volcanism. Evidently these sea mounts and submarine hills along the Western continental margin possibly mark the sites of earlier volcanic activity. The most extensive volcanic activity on the adjacent peninsular India is found in the Deccan Trap period. Agarwal (1975)[1] has given the time span of the Deccan Trap activity from early Paleocene to beyond Eocene (65–34 m.y). Recent studies (Siddiquie and Sukheswala[8], 1976) indicate that the paleocene acid igneous activity on the Laccadive ridge is a westwrad extension of the Deccan Traps. These submarine hills could possibly represent submarine volcanoes along the Western continental slope of the country. At present these observations about volcanism are largely speculative, and therefore, a detailed study involving sampling from these submarine hills would provide an important clue to the Geology and history of the Western margin.

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1. Agarwal, I. K., *Synopsis of Ph.D. Thesis submitted to Bombay University*, 1975.
2. Emery, K. O., Tracey, J. I. Jr. and Ladd H. S., *U.S. Geol. Survey Prof., Paper 260 A* 1954, pp. 265.
3. Fairbridge, R. W., Heezen, B. C. and Tharp M., *Indian Ocaen in the Encyclopedia of Oceanography*, Ed. Fairbridge, R. W, Reinhold Publishing Corporation, New York, 1966.
4. Heezen, B. C. and Tharp, M., *Physiographic Diagram of the Indian Ocean, the Red Sea the South China Sea, the Sulu Sea and the Celebes Sea*, Geol. Soc. Am., Scale 1 : 11,000,000, 1964.
5. Menard, H. W., *Marine Geology of the Pacific*. McGraw-Hill Book Co., New York, 1964.
6. Neprochnov, Iu. P., *Dokl. Akad. Nauk S.S.R.* 1961, 139 (1).
7. Roberts, D. G., Hogg, N. G., Bishop, Derek G. and Flewellen, C. G., *Deep Sea Res.* 1974, 21, 175.
8. Siddiquie, H. N. and Sukheshwala, R. N. *Jour. Geol. Soc. Ind.*, 1976, 17, 539.
9. Uchupi, Elzar, Phillips, J. D. and Prada K. E., *Deep Sea Res.*, 1970, 17, 433.
10. Wiseman, J. D. H. and Ovey, C. D., *Ibid.* 1953, 1, 11.

LETTERS TO THE EDITOR

STUDIES ON PHENOLIC DERIVATIVES OF Ce(IV)

REACTIONS of hexachloroceric acid, H_2CeCl_6 with phenol, resorcinol and pyrogallol yield $(OC_6H_5)\,CeCl_3$, $(OC_6H_4 \cdot OH)CeCl_3$ and $[OC_6H_3(OH)_2]\,CeCl_3$. H_2CeCl_6 was prepared by passing dry HCl gas through ceric hydroxide (2·1 g) suspended in absolute alcohol for 12 hours till the colour changed to deep red. It was filtered to remove undissolved $Ce(OH)_4$. To hexachloroceric acid was added phenol (0·94 g) and the mixture was refluxed till there was no evolution of HCl gas. The solution was filtered and excess of ethanol was removed by distillation under reduced pressure (20–25 mm). It gave a brown paste which on repeated crystallisations from petroleum ether (60–80° C) gave a brown crystalline compound whose analysis is given below:

$(OC_6H_5)\,CeCl_3$

%Ce found 41·0 calc. 41·26; %C found 21·34 calc. 21·22; %H found 1·62 calc. 1·47; %Cl. found 31·45 calc. 31·32.

Other phenolic derivatives of Ce(IV) were prepared by a similar method. The complexes were vacuum dried for 48 hours and analysed for metal, chlorine, carbon and hydrogen. Their analysis corresponded to $(OC_6H_4 \cdot OH)\,CeCl_3$ and $[OC_6H_3(OH)_2]\,CeCl_3$.

Results and Discussion

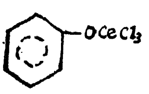
From the analytical data, the reaction can be represented in case of phenol as: $H_2CeCl_6 + C_6H_5OH \rightarrow (OC_6H_5)\,CeCl_3 + 3\,HCl$. A study of the infrared spectra indicates the characteristic aromatic C-H stretching frequency at ~ 3050 cm^{-1}, out of plane C-H bend around 810 and 759 cm^{-1}, C=C frequency at 1650 cm^{-1}, out of plane C—C bend at 690 cm^{-1}, phenolic C—O stretching peak at about 1400 cm^{-1} and the disappearance of hydrogen bonded —OH[1,2] which are relevant to the presence of phenoxy ions. In addition, a high frequency shift (~180 cm^{-1}) of phenolic C—O stretch has been observed in these complexes. The shift in all the compounds is to a higher frequency which is indicative of M—O—C bond formation through oxygen of —OH group of phenols. Out of the three and two hydrogen atoms of the hydroxyl groups present in pyrogallol and resorcinol respectively, only one hydrogen atom has been replaced on complexation with H_2CeCl_6, as confirmed by the i.r. spectra of their derivatives which show bands at ~3620 cm^{-1} due to the presence of free —OH (phenolic) group and at about 1180 cm^{-1} due to —OH deformation. The bands of phenolic group at ~3620 cm^{-1} result from the monomeric structure[3,4]. There is absence of a hydroxyl frequency in the i.r. spectrum of complex with phenol.

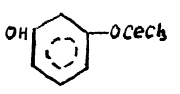
The ultraviolet spectra of phenolic derivatives with recorcinol and pyrogallol display two absorption peaks in 200–350 nm region, *viz.*, around 215 nm ($\log \epsilon_{Max} = 4 \cdot 26$–$4 \cdot 29$) and 280 nm ($\log \epsilon_{Max} = 3 \cdot 78$–$3 \cdot 79$). In the u.v. spectrum of $(OC_6H_5)\,CeCl_3$, these two peaks are observed at 212 nm ($\log \epsilon_{Max} = 4 \cdot 21$) and 270 nm ($\log \epsilon_{Max} = 3 \cdot 6$). The bands around 215 nm are E bands and arise due to ${}^1L_a \leftarrow {}^1A$ transitions and the bands at 270 nm and 280 nm are B-bands and can be assigned to ${}^1L_6 \leftarrow {}^1A$ transitions[5] though in phenolic derivatives with resorcinol and pyrogallol, the two bands are bathochromically shifted with hyperchromic effect by the electron donating substituent —OH group.

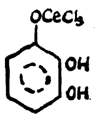
Ebullioscopic molecular weight determination of these complexes carried out in boiling benzene showed them to be monomeric. The molar conductance of these complexes in methanol are very low being of the order of 2–4 $mhos/cm^2$ indicating non-electrolytic nature of the complexes.

All the complexes are found to be diamagnetic. This indicates the absence of free phenoxy radicals which show paramagnetic behaviour. The above studies confirm that in these complexes the covalently bonded chlorine atoms of H_2CeCl_6 have been replaced by the corresponding phenols and these compounds undergo displacement reactions similar to the phenolic derivatives[6] of tricyclopentadienyl and bisindenyl derivatives of Ce (IV).

On the basis of analytical data agreeing with the molecular formulae written above, molecular weight indicating absence of polymeric character, molar conductance showing the presence of non-ionic species and i.r. data giving evidence for the bonding of cerium to oxygen of the phenoxy ions, the diamagnetic behaviour indicating the absence of free phenoxy radicals, the proposed structures of these complexes are:







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1. Bellamy, L. J., *The i.r. Spectra of Complex Molecules*, Methuen, 1964, p. 105.
2. Ueno, K. and Martell, A. E., *J. Phys. Chem.*, 1956, 60, 1270.
3. Silverstein, R. M., Bassler, G. Clayton., and Morrill, T. C., *Spectrometric Identification of Organic Compounds*, Third Edition, John Wiley and Sons, 1974, p. 92.
4. Morrison, R.T. and Boyd, R.N., *Organic Chemistry*, Second Edition, 1969, p. 416.
5. Jaffe, H. H. and Orchin Milton, *Theory and Applications of Ultraviolet Spectroscopy*. John Wiley and Sons,. Inc., 1966, p. 481.
6. Kapur, S., Kalsotra, B. L. and Multani, R. K., *J. Chinese Chem. Soc.*, 1972, 19, 197.

1-NITROSO 2 NAPHTHOL AS A SPRAY REAGENT FOR THE DETECTION OF CANNABIS ON THIN LAYER PLATES

A number of thin layer chromatographic procedures are described for the separation and detection of the active phenolic constituents of cannabis. Chromogenic reagents like fast blue salt B[1], Beam's reagent[2], diazotised *p*-nitroaniline-sodium hydroxide spray[2], blue tetrazolium[3] and Folin-Ciocalteau phenol reagent are generally used to detect the separated phenolic constituents from cannabis. The Folin-Ciocalteau reagent gives blue spots with easily oxidisable substances in addition to phenols. Further, the diazo reagents[4] are found to react with many non-aromatic oxygen compounds to give red colour. Though fast blue salt B is widely used for the detection of cannabis, it was stated in earllier works[5], that this reagent reacts with many compounds other than phenols and can hold no cliam to specificity.

In the present work, therefore, a specific reagent for phenols has been used to detect the phenolic constituents cannabis. Many phenols are known to react with nitroso phenols in its isomeric oxime form in the presence of concentrated sulfuric acid, to give coloured indophenols[6]. Hence 1-nitroso 2 naphthol has been used as a spray reagent for the detection of cannabis and presented in this communication. The reagent is specific for phenols and non-phenolic compounds do not interfere.

Experimental

The cannabis sample has been extracted with three aliquots of benzene[2], which are decanted through a short column of florisil (60-1 The extract was evaporated to a small vol spotted on thin layer plates of silica ge plate is developed in a solvent mixture of chloroform (3 : 7). After the developm chromatograms are air dried and sprayed solution of 1-nitroso 2 naphthol, in con sulfuric acid. On brief heating of the 100° C, the colours are intensified. The are given in Table I. The colours of th 1, 2, 3 change to violet on standing for The reagent has been found to be eff detecting cannabis samples extracted from tures and diluents like starch, jaggery, etc

Table I

Detection of the separated phenolic con cannabis by spraying with 1-nitroso 2 naphth

Spot No.	R_f value	Colour with th spray reagent
1.	0·24	Blue
2.	0·51	Blue
3.	0·67	Blue
4.	0·91	Greyish blue
5.	0·94	Greyish blue

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1. De Faubert Maunder, M. J., *J. Assn Analyst*, 1964, 7, 24.
2. Clarke, E. G. C., *Isolation and Ide of Drugs*, The Pharmaceutic London, 1969 Edition, p. 235.
3. Korte, F. and Sieper, U. H., *J. Ch* 1964, 14, 178.
4. Vegh, A., Szasz, Gy., Brantue; A. and M., *Chem. Abstr.*, 1964, 60, 130
5. Thornton, J. I. and Nakamura, *Forens. Sci. Soc.*, 1972, 12, 461.
6. Feigl, F., *Spot Tests in Organic* Elsevier, Amsterdam, 1966, p. 25

ALKALOIDAL CONSTITUENTS OF *SENECIO SCANDENS*

Senecio scandens is a glabrous, climbing, slightly pubescent plant. Its flowers were examined earlier[1] and found to contain epoxycarotenens and xanthophyll in large amount. Lutein was present in leaves along with β-carotene. But the plant was not examined earlier for the alkaloidal constituents.

The whole plant collected from Darjeeling was extracted with hot ethanol and the extract concentrated to a small bulk after making it acidic with aqueous citric acid. The non alkaloidal constituents were removed by extracting successively with petroleum ether and ether. The aqueous solution was made strongly basic with ammonia and extracted with chloroform. The chloroform extract on evaporation gave a gum which showed positive test for alkaloids but did not show the presence of N-oxides. Further, aqueous solution left after extraction with chloroform contained no N-oxide since on reduction with zinc dust and hydrochloric acid followed by extraction with chloroform did not yield any tertiary amine.

The alkaloidal residue was shown to be a mixture of three by paper and thin layer chromatography. Crystallisation of the residue from ethanol gave a mixture, consisting of two components as shown by paper chromatography using butanol-acetic acid as solvent. It was separated into two constituents (A and B) using cellulose column.

Constituent A showed a spot (R_f 0·56) on paper chromatography, m.p. 236–38° (d), yield 1·5 g, $[\alpha]_D - 55 \cdot 8°$ (*c*, 1·0 mg/ml in chloroform). It gave a picrate m.p. 193–94°. On alkaline hydrolysis[2], it yielded senecic acid lactone m.p. 153–55° and retronecine hydrochloride. These data suggest the alkaloid to be senecionine and it was confirmed by direct comparison with an authentic sample (m.m.p., paper chromatography and infra-red absorption).

The other constituent B had R_f 0·50 (paper chromatography). m.p. 216–18°, yield, 0·7 g. $[\alpha]_D - 138°$ (*c*, 1·27 mg/ml in chloroform). On alkaline hydrolysis it gave seneciphyllic acid (R_f 0·87, m.p. 115–16°) and retronecine hydrochloride (m.p. 161°). It was identified as seneciphyllin by comparison with an authentic sample (m.m.p., paper chromatography and Infra-red absorption).

One of the authors (V.B.) is thankful to CSIR for the research fellowship.

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1. Valden and Mummery, *Phytochemistry*, 1967, 6 (7), 983.
2. Adams and Govindachari, *J. Am. Chem. Soc.*, 1949, 71, 1953.

A NEW TRITERPENE FROM *SWIETENIA MAHOGANII* JACQ.

THE leaves of *Swietenia macrophylla*[1,2,3] are known to contain two interesting terpenoid lactones swietenine, and swietenolide of unusual structure. This encouraged us to study the chemical constituents of the heartwood and leaves of *S. mahoganii* as a part of our general scheme on Indian heartwoods.

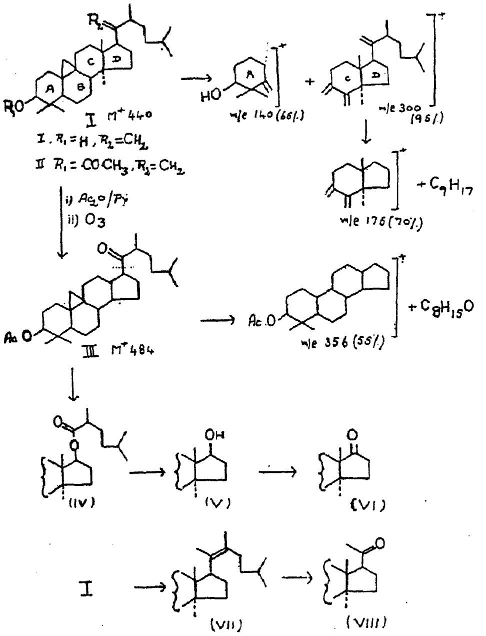
From the leaves, swietenine and stigmasterol glucoside were isolated and identified from their physical and chemical properties. The hexane extract of the heartwood, after extensive chromatography on silica gel, could be separated into six triterpenes, of which β-sitosterol, lupeol benzoate, hederagenin and cycloartenol were identified by comparison with authentic specimens. Of the remaining two triterpenes, one was secured as a major component, (yield: 0·1%), colourless needles from methanol, $C_{31}H_{52}O$, M+ 440, m.p. 145°, $(\alpha)_D + 55°$, (C; 1·0 in $CHCl_3$). L.B. test positive. I.R. 3300 (for hydroxyl), 3040 (for cyclopropane ring) and 890 cm^{-1} (for $= CH_2$). It is characterised by its acetate, colourless plates from $CHCl_3$-MeOH, $C_{33}H_{54}O_2$, m.p. 123°, $(\alpha)_D + 79°$ (C, 1·0 in alcohol). Its physical constants suggested that it could be new and therefore, it is named cycloswietenol (I).

The ^1H NMR spectrum of cycloswietenol (I) as well as its acetate (II) revealed the cyclopropane ring system ($> CH_2$ δ 0·31 *d/d*), and a vinyl methylene at δ 4·72 (*m*) There were 7 methyls between δ 0·92 to 1·1 suggesting that there is an extra methyl in the molecule as in 24-methylene cycloartanol. Hydrogenation of (I) with Pd/charcoal at 30° furnished a dihydro derivative, m.p. 117°, $(\alpha)_D + 21 \cdot 5°$ (C, 1·0 in $CHCl_3$) in which the multiplet resonance at δ 4·72 (for $= CH_2$) disappeared and methyl protons increased by one more methyl. Ozonolysis of II afforded formaldehyde, detected by its dimedone derivative and also by its colouration with chromotropic acid. The corresponding ketocycloswietenol acetate (III) m.p. 98°, $(\alpha)_D + 16°$ (C, 1·0 in alcohol) contained cyclopropane ring system ($> CH_2$, δ 0·31).

The mass spectra of cycloswietenol (I) and its ketone acetate (III) exhibited the characteristic cleavage at ring B to given two fragments m/e 140 (65%) and m/e 300 (95%) indicating the position of the cyclopropane ring system at C_9 and C_{10} as in cycloartanol[4]. The ketoacetate (III) suffered a prominent α-cleavage to give a major fragment, m/e 356 (55%) with loss of $C_8H_{15}O$ (127). Similar loss of the side chain C_9H_{17} (125) was also noticed in cycloswietenol (I) suggesting that the side chain is attached to the C_{17} position in the triterpene. The mass spectra proved unequivocally that cycloswietenol is a tetracyclic triterpene with a long side chain, C_9H_{17}, and a cyclopropane ring on C_9 and C_{10}.

Further evidence to establish the position of the side-chain and also the vinylic methylene group is sought by Baeyer-Villiger oxidation of the keto-acetate (III). Hydrolysis of the resulting product (IV, 80 mg) furnished an alcohol (V, 60 mg). It was later oxidised with Py/CrO_3 to give a ketone (VI, 15 mg) whose I.R. spectrum showed a prominent peak at 1735 cm^{-1} indicating the prtsence of a five-membered ring ketone. The position of the side chain at C_{17} and the ketone function at C_{20}, and consequently the vinylic methylene at C_{20} are thus confirmed.

Only the position of the extra methyl group in the side chain remains to be settled. When (I) was warmed with methanol/HCl, the vinylic double bond migrated to give the isomeric cycloswietenol (VIII) which contained a tetrasubstituted double bond. Its NMR showed two methyls on the double bond (δ 1·72, 6 H) which could be cleaved by ozonolysis. These reactions place the extra methyl at C_{22}. These reactions are taken to confirm tentatively the structure (I) for cycloswietenol. Further work is under progress.



One of the authors (Y. L. N. M.) is grateful to C.S.I.R. for financial assistance. Our thanks are also due to Dr. G. S. Sidhu, R.R.L., Hyderabad, for the NMR and mass spectra.

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1. Chakraborty, D. P., Basak, S. P., Das, B. C and Beugelmans, R., *Phytochemistry*, 1971, 10, 1367.
2. —, Das, B. C. and Basak, S. P., *J. Ind. Chem. Soc.*, 1969, 46 (3), 273.
3. Chakrabartty, T., Connolly, J. D., McCrindle, R., Overton, K. H. and Schwarz, J. C. P., *Tetrahedron*, 1968, 24, 1503.
4. Audier, H. E., Beugelmans, R. and Das, B. C; *Tetrahedron Letters*, 1966, p. 4341.

INFLUENCE OF PHENOLIC COMPOUNDS ON THE GROWTH AND POLYSACCHARIDE PRODUCTION OF *RHIZOBIUM LEGUMINOSARUM*

THE soil polysaccharides, *senso stricto* derived from the microorganisms have been conceded to play a significant role in soil aggregation and stabilization. Of the varied groups of soil bacteria, the symbiotic N_2-fixing *Rhizobia* have been known to produce enormous quantities of polysaccharides and hence play a decisive role in improving the physical properties of soils[2]. However, the growth and activity of rhizobia in soil are more often inhibited by several naturally occurring compounds like phenols, fungistatic substances, etc.[3]. The present paper examines the effect of a few phenolic compounds on the growth and polysaccharide production by *Rhizobium leguminosarum*.

The various phenolic compounds were added at 0·001 M concentration to Waksman No. 77 medium after filter sterilization and dispersed in 100 ml quantities in 250 ml Erlenmeyer flasks. One ml of the *R. leguminosarum* cells experiencing log phase of growth (1 × 10^9/ml), was inoculated into the flasks and incubated on a rotary shaker for 48 hr at 28 ± 1° C. The growth of the organism was measured by determining the biomass. The alkali stable polysaccharide was extracted following the methods of Hassid and Abraham[4] and quantified using anthrone reagent[5]. The constituent sugars of the polysaccharide were identified by paper chromatography using butanol: acetic acid: water :: 4:1:1 u/v solvent system and benzidine-TCA reagent served as the spray solution[6]. The spots were identified by co-chromatography of authentic sugar samples.

The results are set out in Table I. It is of interest to observe that production of polysaccharide had no bearing on cell growth. Among the different phenolic compounds, nitrophenol, rutin, and ferulic acid were more inhibitory than others towards the growth of the organism; it is also revealed that dithiazone, 1,5 dihydroxy anthroquinone and phthallic acid were stimulatory. This observation finds support from the work of Rao and Iswaran[7] and Purushothaman and Balaraman[8] who stated that rhizobia were inhibited by a few phenolic compounds. The polysaccharide production was strongly inhibited by *O*-phenylene-diamine and *para*-amino benzoic acid while nitrophenol, gallic acid and cinnamic acid favoured polysaccharide production.

TABLE I

Effect of certain phenolic compounds on the in vitro *growth and polysaccharide production by* Rhizobium leguminosarum

Phenolic compounds 1	Bio-mass (g)/ 100 ml 2	Quantity of polysaccharides produced (mg/g) 3	Qualtative differences* in polysaccharides produced 4
Nitrophenol	0·13	13·31	Mann, Xyl, Ara
Phloroglucinol	0·31	3·54	Rib, Raff, Xyl
Gallic acid	0·53	13·47	Mann, Xyl, Ara Fru
Quercetin	0·40	1·63	Sucrose, Glu, Rib
8-hydroxy quinoline	0·50	3·87	Fru, Rib, Raff, Ara
Cinnamic acid	0·40	11·59	Glu, Fru, Suc, Lact
Rutin	0·18	3·51	Fru, Xyl, Raff, Rib
Coumarin	0·42	3·29	Glu, Suc, Fru, Xyl
Pyrogallol	0·40	4·18	Xyl, Mann, Ara, Raff, Rib
Para-dichlorobenzene	0·40	7·80	Rib, Raff, Xyl, Mann, Ara, Fru
Hydroquinone	0·39	2·82	Xyl, Mann, Ara, Fru
o-phenylene-diamine	0·50	0·71	Rib, Raff, Xyl, Mann, Ara, Fru
Dithiazone	0·71	0·94	Glu, Rib, Raff, Fru, Xyl
1, 5-dihydroxy anthroquinone	0·68	7·44	Rib, Raff, Xyl, Mann, Ara, Glu Suc, Lact
Salicin	0·51	4·51	Lact, Glu, Suc, Rib
Ferulic acid	0·28	8·66	Rib, Raff, Glu
Benzilic acid	0·53	1·16	Fru, Xyl, Mann, Ara, Rib, Raff
Phthallic acid	0·68	1·41	Suc, Glu, Lac, Xyl
Para-aminobenzoic acid	0·48	0·94	Xyl, Mann, Ara, Fru
Anthranilic acid	0·37	5·03	Rib, Raff, Xyl, Mann, Ara
Tryptophan	0·51	5·36	Fru, Glu, Suc, Lact, Ara
Protocatechuic acid	0·38	7·73	Fru, Rib, Raff
Resorcinol	0·41	7·80	Rib, Raff, Xyl, Mann, Ara
Control	0·59	4·63	Suc, Lact, Glu

*Ara—Arabinose; Lact—Lactose; Rib—Ribose; Fru—Fructose; Mann—Mannitol; Suc—Sucrose; Glu—Glucose; Raff—Raffinose; Xyl—Xylose.

The data reveal that the presence of phenols in the medium greatly influenced the qualitative nature of the polysaccharides. When *R. leguminosarum* was grown in the presence of phenols, the polysaccharide was composed of only hexose sugar units, whereas in the absence of phenols both hexoses and pentoses were discernible. The study of Keele *e. al.*[9] on the polysaccharide composition of several strains of *Rhizobium* revealed the presence of glucose, galactose, rhamnose and mannose in all strains; however, Graham[10] indicated variations in the polysaccharide composition among different strains. No work seems to have been done on the effect of phenols on the polysaccharide composition. As many of the phenols are either reported[11] to be present in soils or released during degradation of organic materials, it is quite possiblr that the soil phenols affect the quantity and quality of the rhizobial polysaccharides *in vivo*. It is clear from the study that the phenolic compounds not only inhibit the growth of *R. leguminosarum* but also alter the polysaccharide composition.

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1. Allison, F. E., *Soil Organic Matter and its Role in Crop Production*, Elsevier Scientific Pub. C., Amsterdam, London, 1973. p. 637.
2. Griffiths, E. and Burns, R. G., *Plant Soil*, 1976, **36**, 599.
3. Alexander, M., *Microbial Ecology*, John Wiley and Sons, Inc., New York, 1971, p. 511.
4. Hassid, W. Z. and Abraham, S., In: Colowick, S. P. and Kaplan, N. O. (ed.), *Methods in Enzymology*, Vol. III, Academic Press, New York, 1957, pp. 34.
5. Scott, A. and Melvin, E. H., *Anal. Chem.*, 1953, **25**, 1656.
6. Block, R. J., Durrum, E. L. and Zweig, C., *A. Manual of Paper Chromatography and Paper Electrophoresis*, Academic Press, New York, 1958.
7. Rao, A. V. and Iswaran, V., *Infektionskrankheiten und Hygiene*, 1973, **2**, 128, 753.
8. Purushothaman, D. and Balaraman, K., *Curr. Sci.*, 1973, **42**, 507.
9. Keele, B., Wheat, R. W. and Elken, G. H., *J. Gen. Appl. Microbiol.*, 1974, **20**, 187.
10. Graham, P. H., *Antonie van Leeuwenhoek*., 1965, **31**, 349.
11. Sinha, M. K., *Plant Soil*, 1972, **37**, 273.

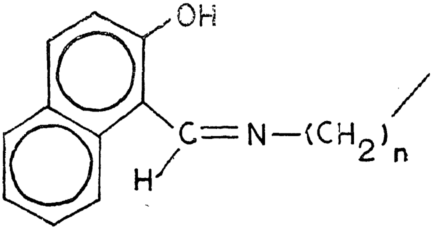
COPPER (II) COMPLEXES OF ONO DONOR TRIDENTATE LIGANDS N-(HYDROXYALKYL)-2-HYDROXYNAPHTHYLIDENEIMINES

THERE has been considerable interest in recent years on the syntheses and magnetic properties of metal (II and III) complexes with tridentate dibasic ligands[1-3]. The tridentate dibasic character of these ligands force the metal (II and III) ions to dimerise or polymerise leading to metal complexes with unusual magnetic and structural properties. We report in this communication the synthesis of copper (II) complexes of ONO donor tridentate dibasic Schiff bases (I) derived from 2-hydroxy-1-naphthaldehyde and alcoholamines, *viz*., ethanolamine, propanolamine and isopropanolamine.

Synthesis of the Complexes

Copper (II) acetate monohydrate (0·005 mole) was dissolved in 60 ml of hot methyl alcohol. To this a methyl alcoholic solution (45 ml) of the appropriate condensed Schiff base (0·005 mole) was added and the mixture was refluxed for 2 hr. The green precipitates formed were filtered under reduced pressure, washed with methyl alcohol and dried under vacuum. The complexes are insoluble in common non-coordinating organic solvents. The analytical and characterisation data of the complexes are presented in Table I.

The data indicate 1:1 metal: ligand ratio in the complexes. The infrared spectra of the complexes do not exhibit the ν(OH) stretch and this is indicative of the dibasic behaviour of the ligands in the complexes. The magnetic moments of the complexes, copper (hydroxynaphthaldehyde-ethanolamine) and copper (hydroxynaphthaldehyde-isopropanolamine) determined by the Gouy method, increase as the temperature is lowered from 297 to 83° K. The exchange integral, J of the complexes calculated using the Bleaney-Bowers equation[4] is + 67 and + 28 cm^{-1} respectively. The positive J values and the increase of the magnetic moment with lowering of temperature are indicative of the presence of ferromagnetic exchange in these complexes[5,6]. The magnetic moment of the complex copper (hydroxynaphthaldehyde-propanolamine) is 0·46 B.M. at 297° K and the moment shows little temperature dependence. J of this complex is $-846\ \text{cm}^{-1}$. The negative J value and low magnetic moment are indicative of the presence of antiferromagnetic exchange in this complex[1]. This complex is a novel example where the spins of the interacting copper (II) ions are completely coupled having a sole population of the diamagnetic singlet state. Only few such copper (II) complexes with sole population of the singlet ground state have been reported in the literature[7]. The magnetic properties of Cu (hydroxynaphthaldehyde-propanolamine) and the other two complexes containing ethanolamine and isopropanolamine are different and it is likely that the structures of the antiferromagnetic and ferromagnetic complexes are different[1].



Ia. n = 2, 3.

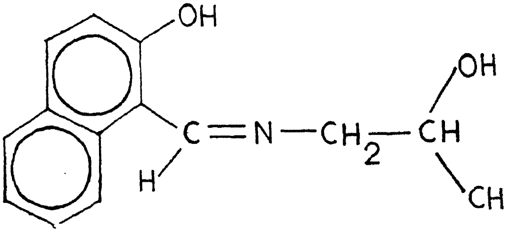


TABLE I

The analytical and characterisation data of copper (II) Schiff base complexes[a,b]

Complex		%Cu	%N	Temp. (°K)	χ_M^{corr} (10^{-6} cgs unit)	μ_{eff} (B.M.)	J^c cm^{-1}
Cu (hydroxy-ethanolamine)	Found:	22·1	4·9	297	1489	1·89	+67
$CuC_{13}H_{11}NO_2$	Reqd.:	22·96	5·06	155	2998	1·94	
				85	5805	2·01	
Cu (hydroxy-propanol-amine)	Found:	21·0	5·2	297	88	0·46	−846
$CuC_{14}H_{13}NO_2$	Reqd.:	21·86	4·82				
Cu (hydroxy-isopropanol-amine)	Found:	21·0	4·9	297	1352	1·84	+28
$CuC_{14}H_{13}NO_2$	Reqd.:	21·86	4·82	118	3702	1·88	
				83	6048	2·01	

a Abbreviation: hydroxy = 2-hydroxy-1-naphthaldehyde.

b The magnetic moment was calculated using the Curie equation: $\mu_{eff} = 2\cdot 84\ (\chi_M^{corr} \times T)^{1/2}$ B.M.

c Temperature independent paramagnetism term = 60×10^{-6} cgs. unit, $g = 2\cdot 1$.

The synthesis and characterisation of copper (II) complexes using other alcoholamines like 2-amino-1-butanol, 4-amino-1-butanol and 2-amino-2-methyl-propanol are in progress. The corresponding oxovanadium (IV) complexes are also being studied in order to compare the magnetic properties of these $s = \frac{1}{2}$ systems.

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1. Hatfield, W. E. and Whyman, R., *Transition Metal Chemistry*, 1969, **5**, 47.
2. Bertrand, J. A., Breece, J. L., Kalyanaraman, A. R., Long, G. J. and Baker, Jr., W. A., *J. Amer. Chem. Soc.*, 1970, **92**, 5233.
3. — and Eller, P. G., *Inorg. Chem.*, 1974, **13**, 928.
4. Bleaney, B. and Bowers, K. D., *Proc. Roy. Soc. Ser. A.*, 1952, **214**, 451.
5. Casey, A. T., Hoskins, B. F. and Williams, F. D., *Chem. Comm.*, 1970, p. 904.
6. Barnes, J. A., Hodgson, D. J. and Hatfield, W. E., *Inorg. Chem.*, 1972, **11**, 144.
7. Harris, C. M., Hoskins, B. F. and Martin, R. L., *J. Chem. Soc.*, 1959, p. 3728; Gupta, S., Katla, K. C. and Chakravarty, A., *Inorg. Chem.*, 1971, **10**, 1534; Majumdar, A. K. and Saha, S. C., *J. Indian Chem. Soc.*, 1973, **50**, 697.

CHEMICAL EXAMINATION OF *TECOMELLA UNDULATA* (G. DON) SEEM

Tecomella undulata (G. Don) Seem (Bignoniaceae) is described to be useful[1,2] in curing urinary discharges, enlargement of spleen, gonorrhoea, leucoderma and liver diseases. Its bark has been the subject of extensive chemical study[2-4]. In continuation of our earlier work[5,6] on extractives from its heartwood, we now report the isolation and identification of veratric acid; which is the first report of its occurrence in free state from the heartwood of a tree.

Air dried and coarsely powdered heartwood (550 g) was successively extracted with petroleum ether 60–80° and acetone. A good yield of lapachol[5] was obtained from petrol extract. Acetone extract on concentration and keeping in refrigerator gave a solid (tecomelloside)[6]. Latter was removed by decantation. From the mother liquor, solvent was completely removed and the deep orange-red mass obtained was separated into ether-soluble (1·34 g) and ether-insoluble (3·15 g) fractions. The insoluble fraction contained mainly the glucoside. The ether-soluble fraction resisted crystallisation and therefore was subjected to column chromatographic resolution over diactivated silicagel. From ethylacetate-chloroform (2 : 3 and 1 : 1) fractions, after removal of solvent an orange-yellow solid (0·49 g) was obtained and was further purified by filtration of its chloroform solution through a column of silicagel and then crystallised from ethyl acetate-petroleum ether 60–80° (1 : 1) as needles, m.p. 181–182° (Found: C, 59·45; H, 5·63; OCH_3, 34·20; Calcd, for

$C_9H_{10}O_4$: C, 59·34; H, 5·53; OCH_3, 34·06%). It gave acidic reactions and showed ν_{max} (KBR) : 2778–2500 (OH)[7], 1684 (C=O), 1503, 1520, 1475 (Ph), 1272 (C=O) and 926 cm^{-1} etc; λ_{max} (EtOH) : 222, 258, 290 mμ; NMR signals ($CDCl_3$, δ) : 13·21 (s, COOH, 1 H), 4·06 (s, 2 OCH_3, 6H); aromatic protons 7·8 (1H, d, J=2 cps), 7·1 (1H, d, J=9 cps) and 8·0 (1H, dd, J=9 and 2 cps); MS (m/e) : 182 (M^+), etc. These data indicated the compound to be veratric acid; confirmed by co-TLC, m.m.p. and by preparation of methyl ester (CH_2N_2), m.p. 57–58° (Reported[11] 59–60°).

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---

1. Chopra, R. N., Nayer, S. L. and Chopra, I. C., *Glossary of Indian Medicinal Plants*, CSIR, New Delhi, 1956, p. 240.
2. Pandey, V. B. and Dasgupta, B., *Experientia*, 1970, 26, 1187.
3. — and —, *J. Indian Chem. Soc.*, 1971, 48, 937.
4. Joshi, K. C., Singh, P. and Prakash, L., *Phytochemistry*, 1972, 11, 1498.
5. — and —, *Ibid.*, 1974, 13, 663.
6. —, — and Prakash, L., *IUPAC Abstracts*, 1972, p. 163; *Phytochemistry*, 1975, 14, 1441.
7. Pauling, L., *Nature of the Chemical Bond*, O.U.P., 1940, p. 306.
8. Hirshberg, Y., Lavie, D. and Bergmann, E. D., *J. Chem. Soc.*, 1951, p. 1030.
9. Hergert, H. L., *J. Org. Chem.*, 1960, 25, 405.
10. Hodgkin, J. H., Craigie, J. S. and MacInnes, A. G., *Can. J. Chem.*, 1966, 44, 74.
11. Heilbron, I. and Bunbury, H. M., *Dictionary of Organic Compounds*, Eyre & Spottiswoode, London, 1953, 4, 662.

RECORD OF PERMIAN AMMONOID *CYCLOLOBUS* FROM THE TILEL AREA, BARAMULLA DISTRICT, KASHMIR

THE authors place on record the discovery of *Cyclolobus walkeri* (Diener, 1903)[1], a characteristic index fossil of uppermost Permian in the Tilel area of North Kashmir. The fossil specimen was collected from a fallen block of a compact shaly slate in the Badogam Nala (34° 34′ : 75° 04′) of Tilel area, Baramulla District. The Badogam Nala drains a predominently Salkhala country which is overridden by Dras Volcanic belt further north. However, this find indicates the existence of patches of marine Upper Permian, probably as infolds, within the Salkhala. Such occurrences of Permian have been reported further NW in the Burzil valley[3].

Cyclolobus is a comparatively rare genus in the Permian of the Himalayas. The present specimen (Figs. 1 and 2) is characterised by large shell, planispiral, involute, almost flat flanks, whorl section almost lanceolate ventrolateral shoulders and venter rounded, and small umbilicus.

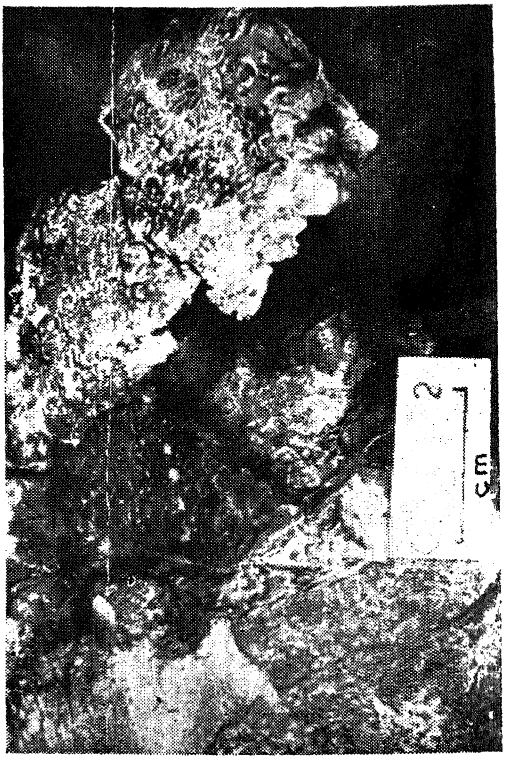

FIG. 1

Furnish *et al.*[2] reported *Cyclolobus walk* (Diener, 1903) from the Guryul Ravine sectio in Kashmir. Our specimen bears close resemblance to this specimen in having almost fl flanks, rounded ventrolateral shoulders, venter wit small umbilicus and identical sutural details However, the present specimen differs from th Guryul Ravine specimen in having constriction upto a conch diameter of 32 mm.

The horizon yielding *Cyclolobus walkeri* ca be correlated with the top of Zewan formation o Kashmir, the upper Kuling shale of the Centr Himalayas, the Upper Chhidru formation of th Salt Range and its Ankitohazo beds of Madagascar

All the above occurrences have been interpreted by Furnish *et al.*[2] as representing the Chhidruan stage of the middle Ozhulfian Series.

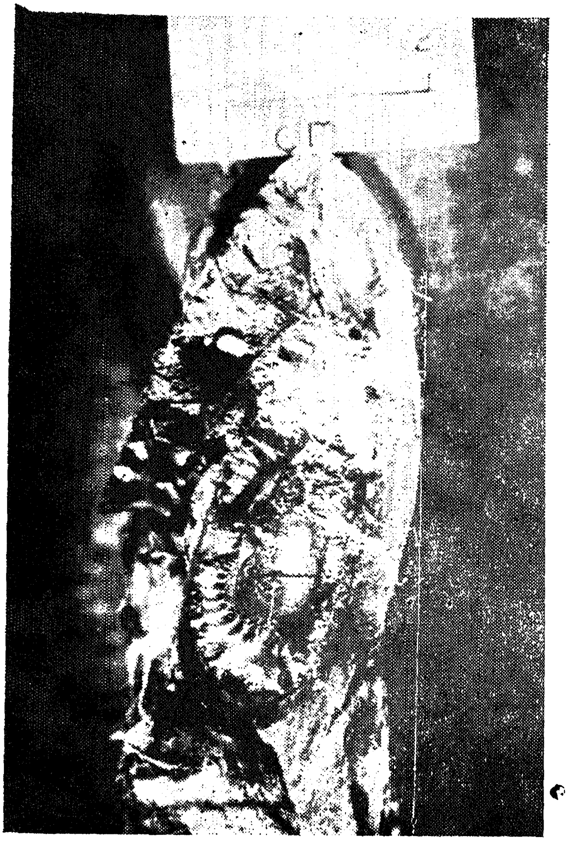

FIG. 2

The authors are grateful to Shri H. M. Kapoor, Geologist (Sr.), G.S.I., Lucknow, for assistance in identification. They are also indebted to Shri M. N. Sehgal, Director, Geological Survey of India, Srinagar, for valuable discussions and guidance.

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---

1. Diener, C., *Palaeont. Indica*, Ser. 15, 1903; 1 (5), 204.
2. Furnish, W. M., Glenister, F., Nakazawa, K. and Kapoor, H. M., *Science*, 1973, 180, 188.
3. Pascoe, E. H., *A Manual of the Geology of India and Burma*, 1968, 2, 808.

DIURNAL RHYTHMIC ACTIVITY OF ALKALINE PHOSPHATASE IN THE SCORPION, *HETEROMETRUS FULVIPES*, C. KOCH.

A REVIEW of the literature has shown that dehydrogenases[1,2] and esterases[1] exhibited diurnal rhythmic activity in the scropion, *Heterometrus fulvipes*, C. Koch. In view of the existence of diurnal variations in the scorpion, it is of interest to find whether such changes would also occur in the activity of alkaline phosphatase (EC 3·1·3·1), an enzyme that catalyzes the hydrolysis of phosphamides and compounds other than simple orthophosphoric acid monoesters[3]. Hence an attempt has been made to study the activity of alkaline phosphatase in different tissues of the scorpion.

The hepatopancreas, heart, pedipalpal muscle and nervous tissue were isolated from the live scorpions of similar size at six different timings of the day (*viz.*, 08·00, 12·00, 16·00, 20·00, 00·00 and 04·00 hrs.) The tissues were immediately transferred to prechilled glass tubes and 2% (w/v) homogenates were prepared in 0·25 M ice-cold sucrose solution and centrifuged at 2500 rpm for 10 min; 0·4 ml of each supernatant, containing 8 mg. of tissue, was assayed for the alkaline phosphatase activity by the method of Fiske and Subba Rao as given by Oser[4].

Figure 1 indicates that, in general, the enzymic activity on the basis of gram weight of tissue followed the order hepatopancreas > heart > pedipalpal muscle > nervous tissue. The enzyme exhibited maximal activity at 20·00 hr. and minimal activity at 08·00 hr. of the day (Fig. 1). The higher levels of enzymic

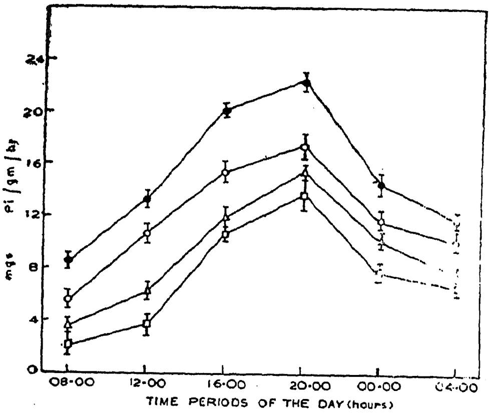

FIG. 1. Diurnal rhythmic activity of alkaline phosphatase in different tissues of the scorpion. (Values, expressed as mgs pi/gm/hr, are mean ± S.D. of five observations.)

●—● Hepatopancreas; ○—○ Heart;
△—△ Pedipalpal muscle and
□—□ Nervous tissue.

activities might be due to the increased locomotor activity[5], and metabolism[2]. Morover, these results are in agreement with the earlier investigations on dehydrogenases[1,2] and esterases[1]. Based on these observations it may be suggested that the alkaline phosphatase activity followed a regular circadian rhythm.

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1. Devarajulu Naidu, V. and Padmanabha Naidu, B., *Ind. J. Exp. Biol.*, 1976, **14**, 1.
2. Venkateswara Rao, P. and Govindappa, S., *Proc. Ind. Acad. Sci.*, 1967, 66 B (6), 243.
3. Burstone, M. S. In: *Enzyme Histochemistry and its Applications in the Study of Neoplasms*, Academic Press, New York and London, 1962, p. 166.
4. Oser, B. L., *Hawk's Physiological Chemistry*, McGraw-Hill Book Company, New York, 1965, p. 636.
5. Gopalkrishna Reddy, T., *Thesis*, Sri Venkateswara University, Tirupati, 1967.

A COMPARATIVE STUDY OF THE EFFECTS OF PALMITATE AND ACETATE FORMS OF VITAMIN A ON TOAD TADPOLES

VARIOUS forms of vitamin A given in excess differ in their toxicity and biological effects produced[1-3]. In studies on amphibians also different forms of this vitamin are found to vary in their effect[1]. The present study was undertaken to compare the effect of the two esters of vitamin A, palmitate and acetate, on the tadpoles of *Bufo andersonii* Boulenger of identical age in equal periods of time. Two age groups of the tadpoles, 4 and 7 days old after hatching from the same spawn, were divided into groups of 50 individuals each. One group each was reared in solutions containing 1, 5, 10, 15, 20 and 30 I.U./ml of vitamin A palmitate (Arovit-Roche) and vitamin A acetate (Glaxo) and in tap water (controls). Known quantities of the vitamins were dissolved in small amounts of ethanol and the solutions were then diluted with water to the required concentrations. The animals were transferred to fresh media every two days and fed maximally with boiled spinach. The experiments carried out at room temperature lasted for 10 days in each case.

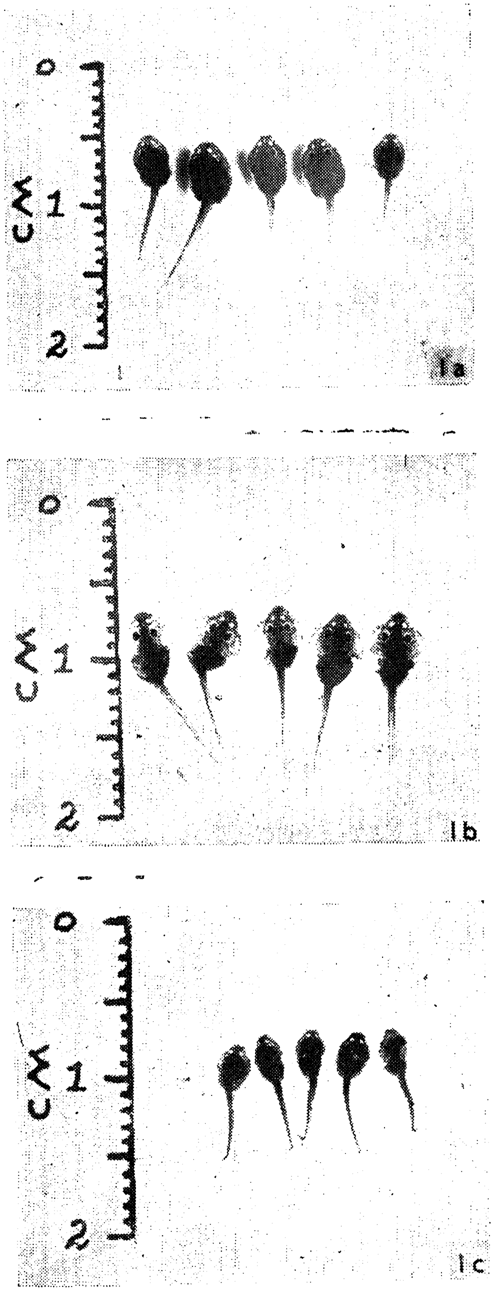
The numbers surviving on the 10th day in each experiment are shown in Table I. The older tadpoles were in general much more tolerant to the toxic effects of the two vitamins as compared to the younger ones. However, the acetate was far more toxic than the palmitate for both age groups. Even in 1 I.U./ml solution of the acetate only 72 to 76% of the two age groups survived the 10 days treatment and in concentrations of 15 I.U./ml and higher all or nearly all died during the same period. On the contrary, in all palmitate treated groups of 7 days old tadpoles the least number surviving in even the highest concentration of the drug was still as high as 74%. Even among the younger larvae the survivors on 10th day were less than 50% when the palmitate concentration was 20 I.U./ml; but the next higher concentration of this form also was extremely lethal to this age group. Most mortality occurred earlier in the acetate than in the palmitate treated groups.

TABLE I

Percentage of Bufo andersonii *tadpoles surviving after 10 days of treatment with vitamin A palmitate and vitamin A acetate*

Rearing medium	% of survivors on Day 10			
I.U. of vitamin A/ml	4 days old Palmitate treated	tadpoles Acetate treated	7 days old Palmitate treated	tadpoles Acetate treated
Nil (water—controls)	100		100	
1	100	72	100	76
5	100	42	96	50
10	100	2	98	18
15	96	0	84	2
20	44	0	82	0
30	4	0	74	0

The two esters affected the tadpoles in various other ways also but not identically. Growth of the larvae was retarded by both but it was particularly noticeable in the tadpoles treated by even low concentration, 5 I.U./ml, of the acetate (Fig. (1a–c) which also caused severe haemorrhage in the head region and curving of the tail. Both acetate and palmitate produced general oedema and some depigmentation particularly of the head and branchial regions. However, while these changes occurred in tadpoles at high concentrations (15 I.U./ml and more) of palmitate, even low amounts of acetate produced similar effects; but higher concentrations of the acetate did not cause any noticeable changes of this nature. The tadpoles treated with 15–30 I.U./ml palmitate secreted large quantities of mucas which was found oozing out from the cloaca, spiracle and mouth (Fig. 2) but this did not occur in any of the acetate treated larvae.



FIGS. 1*a–c*. Tadpoles reared for 8 days in (*a*) water (controls) and in 20 I.U./ml solutions of (*b*) vitamin A palmitate and (*c*) vitamin A acetate from 4 days after hatching.

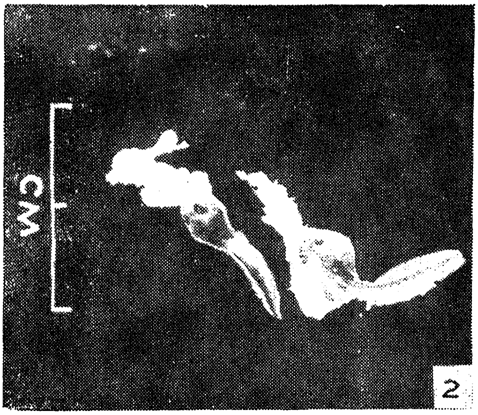


FIG. 2. Mucus emerging from the mouth of tadpoles treated with 30 I.U./ml vitamin A palmitate.

The excessive mucus production in palmitate larvae must have been due to hyperplasia of the mucosal cells of the gastro-intestinal tract caused by the excess of vitamin A as has also been reported for *Xenopus* larvae fed vitamin A alcohol or acid[1]. That this did not happen in the larvae given acetate may be attributed to the greater toxicity of this form which, even in small amounts, perhaps killed the cells rather than causing mucous hyperplasia. Vitamin A acetate has been reported to inhibit mucus secretion from even the normal mucoid epithelia in organ culture[4].

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1. Weismann, G., Bell, E. and Thomos, L., *Amer. J. Path.*, 1963, 42, 571.
2. McLean, F. C. and Budy, A. M., in *Vitamins and Hormones*, Ed. R. S. Harris *et al.*, Vol. 21, Academic Press, 1963, p. 51.
3. Thompson, J. N. and Pitt, G. A. T., *Nature*, (London), 1960, 188, 672.
4. Fell, H. B. and Mellanby, E., *J. Physiol.*, 1953, 119, 470.

VARIABLE SEX RATIO IN SOME HIGH ALTITUDE LIZARDS FROM KASHMIR

AMONG the lizards in general, a normal 1 : 1 sex ratio has been reported[1,2]. But the fluctuations with regard to this ratio have also been pointed out in some species[3,4]. The population structure of *Agama tuberculata* (oviparous) and *Lygosoma himalayanum* (ovoviviparous); the two commonly occurring high altitude lizards in Kashmir has been studied.

Live specimens of both sexes of the species were collected from their habitats in the valley of Srinagar (*A. tuberculata*, elevation of 1750–1900 m and *L*

TABLE I

Incidence of sex ratio in A. tuberculata *and* L. himalayanum

Month	*Agama tuberculata*		*Lygosoma himalayanum*	
	1972/73	1973/74	1972/73	1973/74
April	1·14±0·24	0·84±0·21	1·33±0·22	1·25±0·26
May	1·38±0·23	1·09±0·23	1·33±0·23	1·75±0·28
June	1·44±0·22	1·20±0·23	0·86±0·21	1·25±0·28
July	0·93±0·22	1·00±0·24	0·80±0·24	1·38±0·30
August	1·72±0·25	1·60±0·23	1·25±0·24	0·95±0·24
September	1·66±0·25	0·87±0·21	1·29±0·24	0·87±0·24
October	1·25±0·24	1·14±0·25	1·61±0·26	1·60±0·27
November	1·22±0·23	1·30±0·22	1·28±0·26	1·19±0·25
December	1·28±0·23	1·31±0·23	..	..
	AV. SD = ± 0·23	Av. SD = ± 0·22	Av. SD = ± 0·24	Av. SD = ± 0·2

himalayanum, elevation of 2280–2590 m.). Five batches of ten lizards each were taken each month, excluding the months of hibernation, December to April, from 1972 to 1974. The standard deviation (SD) and coefficient of variation (cv) were calculated.

While *A. tuberculata* is a rock loving lizard; *L. himalayanum* dwells in the grassy uplands and alpine meadows, where they are seen from late April to November end, when the first snowfall starts.

The data of sex ratio of the species are presented in Table I. The overall sex ratio of male : female was 1·4 : 1 (SD ± 0·22) in *A. tuberculata* and 1·19 : 1 (SD ± 0·25) in *L. himalayanum*, as against an expected sex ratio of 1:1[1,2]. The cause of the deviation from the expected ratio in our catches could not be ascertained nor does such a deviation appear to be uncommon in lizards[4,5].

The present finding, about the outnumbering of the females by males got a further support in having a small coefficient of variation (17·1 and 19·1 in *A. tuberculata* and 19·7 and 21·0 in *L. himalayanum*); thereby showing the consistency, uniformity and stability of the variation.

On the whole the deviation in the sex ratio in *A. tuberculata* seems to be of reverse order when compared to *Calotes numericola*[4], wherein females are reported to be far more numerous than males. Church[5] explains this difference as due to the general alertness and quickness of males in escaping capture, although his own subsequent observation of these lizards from a different area, as also those of Suba Rao *et al.*[4] show that more males are captured than females

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---

1. Ishihara, S., *Bull. Kyoto. Gakugei. Univ.*, Ser E, 1964, **25**, 27.
2. Telford, S. R., *Copeia*, 1969, **3**, 548.
3. —, *Ibid.*, 1971, **4**, 670.
4. Suba Rao, M. N. and Rajabai, B. R., *Herpetologica*, 1972, **28**, 285.
5. Church, G., *Copeia*, 1962, **2**, 262.

ON THE OCCURRENCE OF LYMPHOCYSTIS IN *ANABAS TESTUDINEUS* (BLOCH)

DURING the course of cage culture experiments of air-breathing fishes in floating nylon net cages installed in a sewage-fed pond situated in Darbhanga township (26·12 N, 85·53 E), a single specimen of *Anabas testudineus* developed peculiar proliferous growths of binding tissue on the fins as well as on the body proper. The most heavily affected part was the tip of the fins, particularly the caudal (Fig. 1). The infected specimen was a male and measured 130 mm in total length and 64 g in total weight. The growth of tissues in the infected portions looked like specks of cauliflower. From the characteristic features, the disease appeared to be Lymphocystis.

Wolf[11] has stated that Lymphocystis is a unique, benign, giant cell disease of world-wide distribution in freshwater and marine teleosts of relatively advanced evolutionary status. Various theories have been presented for Lymphocystis by several workers[1,2,7-9,12]. However, Walker[5] and more recently Walker and Weissenberg[6] demonstrated that these bodies were filled with virus particles when viewed under the electron microscope.

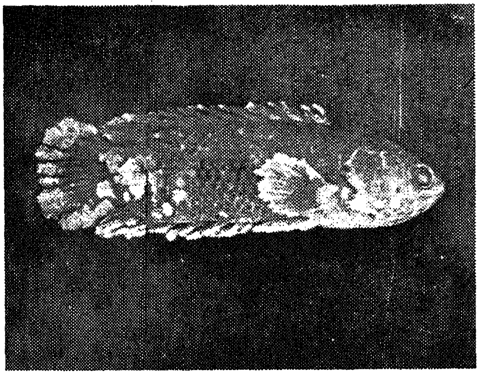

FIG. 1. Lymphocystis growth on the fins and body of *Anabas testudineus.*

There is no earlier report available on the occurrence of this disease in *Anabas testudineus.* Its occurrence has, however, been reported in other species[3,4,7,10]. The disease is known to be highly infectious but there is little information about the mode of its transmission.

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1. Awerinzew, S., *Zool. Anz.*, 1907, 31, 881.
2. Sandeman, G., *Ann. Rept. Scott. Fish Board,* 1892, 11, 391.
3. Shelton, R. G. J. and Wilson, K. W., *Aquaculture,* 1973, 2, 395.
4. Van Duijn, Jr. C., *Disease of Fishes,* Water life, Dorset House, London, 1956, p. 112.
5. Walker, R., *Virology*, 1962, 18, 503.
6. — and Weissenberg, R., *Ann. N.Y. Acad. Sci.*, 1965, 126, 375.
7. Weissenberg, R., *Sitzungsber. Kgl. Preuss. Acad. Wiss, Sitz. Physik. Mathem.*, Jg., 1914, p. 792.
8. Weissenberg, R., *Biol. Bull.*, 1939, 76, 251.
9. —, *Zoologica,* 1939, 24, 245.
10. —, *Ibid.*, 1945, 30, 169.
11. Wolf, K., *Symp. Zool. Soc. Lond.*, 1972, 30, 305.
12. Woodcock, H. M., *Rep. Lancs.*, Sea-Fish. Labs., 1904, 12, 63.

STUDIES ON PROTEASE PRODUCTION IN *PENICILLIUM JAVANICUM* VAN BEYMA

Penicillium javanicum Van Beyma P×26 elaborates a proteolytic enzyme. The optimum production of which could be induced with 10 days incubation at 25° C. Presence of glucose in the medium completely inhibits enzyme production.

Although the strain *Penicillium javanicum* displays a variety of biosynthetic activity and produces antibiotics[7], pigments[2], fat[5], etc., no studies have yet been done with regard to the abilities of the organism to elaborate proteolytic enzyme.

To find out a suitable nutritional environment conducive to steady accumulation of the enzyme the strain *P. Javanicum,* P×26 was grown in Czapek-Dox[1] medium where glucose was replaced by 3% wheat bran (WB), 1% wheat flour (WF), 3% rice bran (RB) and 0·1% Bovine Serum Albumin (BSA) separately. Thirty ml of each medium was taken in 100 ml capacity Erlenmeyer flask and was inoculated with conidial suspension having a concentration of $12 \cdot 0 \times 10^6$ (approx.) and incubated at 25° C for 10 days in the case of BSA and 5 days in the case of others, before the respective mycelia were collected. Ten replicates for each medium were considered.

Wheat bran-Czapek-Dox medium (WB-CD) was found to be the most suitable for protease production and was selected for further study. The flasks containing inoculum were kept at different temperatures, 15°, 20°, 25°, 30° and 35° C and the enzyme assay was done on the 5th day. Optimum incubation period of 10 days was determined by observing the accumulation of enzyme on 5th, 10th, 15th, 20th and 25th day at 25° C.

The effect of glucose on the production of enzyme was studied by adding glucose at different concentrations (1, 5, 10, 15 and 20%) to WB-CD medium and noting the result on the 5th and 10th days. The initial pH of the medium was adjusted to 6·5. One flask without glucose was taken as the control.

The enzyme reaction was carried out in 1 ml of 1·5% milk casein in 0·15 M phosphate buffer at pH 7·3 with 1 ml of culture filtrate and 1 ml ethylene—diamine tetraacetic acid (0·458 g EDTA dissolved in 100 ml of water) at 30° C for half an hour. The reaction was stopped by the addition of 1·0 ml of 20% TCA. Precipitate was then removed by filtration. After filtration, 0·6 ml of the filtrate was taken in a tube and 4 ml of protein reagent added to it. After 10 minutes, 0·4 ml Folin reagent was added to the mixture which was left for 30 minutes at room temperature. Colour of the enzyme assay mixture was read with a Bausch and Lomb Spectronic 20 at 540 nm against water blank. All the readings were corrected with reference to the values of blanks prepared by adding TCA at 0 minute in the reaction mixture containing enzyme solution, milk casein and EDTA. Unit activity of alkaline protease is expressed as the amount of enzyme that yields a change in optical density at 540 nm equivalent to 1 μg of Bovine Serum albumin per 30 min at 30° C.

Of all the substrates tried wheat bran was found most suitable for protease production (Table I). Variation in proteinase production with fermentation medium has also been reported in other fungi[3,6,8]. Wang and Hesseltine[11] reported in a more or less similar situation that proteolytic activity of *Rhizopus* was higher in wheat flour medium than in other media.

TABLE I

Effect of substrate on mycelial growth and enzyme production

Substrate	Mycelial wt. (mg)	Unit production of enzyme
Wheat bran	250·00	14·5
Wheat flour	350·00	10·2
Rice bran	150·25	5·0
Bovine Serum Albumin	100·15	..

The difference in the behaviour of the organism was ascribed as partially due to differnce in the C/N ratio of the medium. It was also found by the same authors that proteolytic activity of *R. oligosporus* reached its maximum at 72-96 hours of incubation. Similar result was reported with other organisms[3,8,10] although with *Mucor pusillus*[10] the incubation temperature for optimum production was 35°C. However, the incubation time requried for optimum production of enzyme in *P. javanicum* is rather long extending 10 to days at 25° C (Figs. 1 and 2).

Epstein and Beckwith[4] reported that formation of inducive enzyme is often strongly inhibited when the bacteria have alternative energy source such as glucose. Sing *et al.*[9] showed that protease production in an *Aspergillus niger* mutant is inhibited in the presence of gluscose in the medium. A similar situation observed by Somkuti and Babel[10] in *Mucor pusillus* was explained as due to accumulation of organic acid in the medium with subsequent lowering of pH. But in the present investigation this does not appear to be the reason as the pH was acidic (4·5–5·0) on the 5th day and increased to 8·5 on the 10th day whil there was no change of proteolytic acitvity.

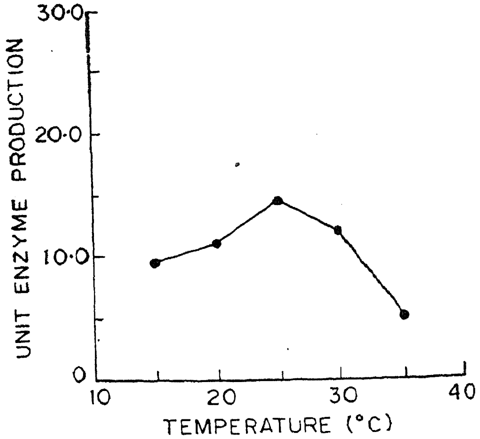

FIG. 1 Effect of incubation period on prot production in *P. javanicum*.

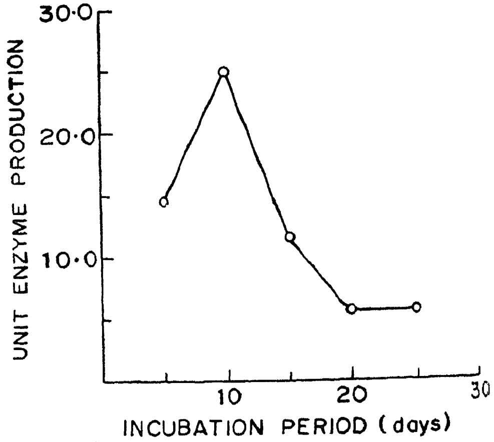

FIG. 2 Effect of temperature on protease produc in *P. javanicum*.

The gift of the strain, *P. javanicum* Px26 by P B. S. Mehrotra, University of Allahabad, gratefully acknowledged. This investigation is supp ted by C.S.I.R., New Delhi, India.

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1. Basu, S. N., Bose, R. G. and Bhattacharya, J. *J. Scient. Ind. Res.* (*India*), 1955, **14 C**,
2. Bhattacharya, M. and Chaudhuri, K. L., *Proc. Ind. Sci. Congr. III*, 1973, No. 140, p. 34
3. Dworschack, R. G., Koepsell, H. J. and Lag A. A., *Arch. Biochem. Biophys.*, 1952, **41**,
4. Epstein, W. and Beckwith, J. R., *Ann. Biochem.*, 1968, **37**, 411.

5. Gray, W. D., *The Relation of Fungi to Human Affairs*, Henry Holt and Company, Inc., New York, 1959.
6. Johnson, A. G. and Martin, S. M., *Agr, Biol. Chem.*, 1964, **28**, 734.
7. Mukherjee, M. and Chaudhuri, K. L., *Sci. and Cult.*, 1975, **41**, 548.
8. Oleniacz, S. W. and Pisano, M. A., *Appl. Microbiol.*, 1968, **16**, 96.
9. Sing, D. P., Sing, R. and Vyas, S. R., *Indian J. Microbiol.*, 1975, **15** 109.
10. Somukuti, G. A. and Babel, F. J., *Appl. Microbiol.*, 1967, **15**, 1309.
11. Wang, H. W. A. L. and Hesseltine, C. W., *Can. J. Microbiol.*, 1965, **11**, 727.

PASSION FLOWER MOSAIC VIRUS DISEASE A NEW RECORD FOR INDIA

THE genus Passiflora consists of over 300 species of herbaceous and woody climbers, occasionally shrubs or small trees. *Passiflora caerulea*, *P. foetida*, *P. edulis*, *P. alba*, *P. alatocaerulea*, *P. trifasciata*, *P. van volexmi*, *P. holosericea*, *P. laurifolia*, *P. decaisneana*, *P. murucuia*, *P. quadrangularis*, *P. racemosa* and *P. raddiana* are commonly cultivated in India. Passion flower (*P. caerulea*) plants growing in the University campus were found to be affected by virus-like disease with 70–80% incidence.

The symptoms observed on young foliage of diseased plants were dark green mosaic mottling, puckering or blistering, malformation and distortion of leaves. On older leaves the symptoms of chlorotic ring spots and mottling was evident, no malformation or distortion of the older leaves was observed. (Fig. 1). In the case of severe infection the plants were considerably stunted.

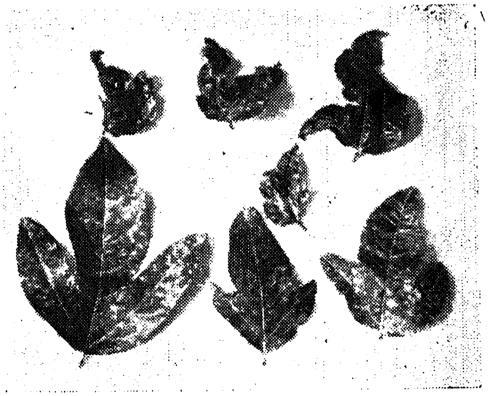

FIG. 1. Symptoms of passion flower mosaic virus disease on *Passiflora caerulea*.

Transmission studies by sap inoculation and budding were undertaken to prove the viral nature of the disease in question. Sap inoculation was done by macerating the leaves of passion flower displaying typical symptoms of moaic with 0·05 M phosphate buffer (pH 7·5) by pestle and mortar. The sap was inoculated on the young leaves of *Gomphrena globosa* and *Chenopodium amaranticolor* by cotton swab method. Necrotic local lesions were produced on the leaves of *G. globosa* after about 15 days of inoculation while chlorotic local lesions were developed on *C. amaranticolor* after 8 days of inoculation. This disease was also transmitted by budding and approach grafting. In about 25–30 days after budding the leaves of new sprouts arising from the budding of *P. edulis f. flavicarpa* exhibited typical symptoms of mosaic (Fig. 2). The seedlings of *P. edulis f. flavicarpa* for graft transmission purpose were grown from heatlhy seeds in earthen pots containing steam sterilized soil with compost mixture. In the case of approach grafting, the typical symptoms of mosaic developed after 30–35 days.

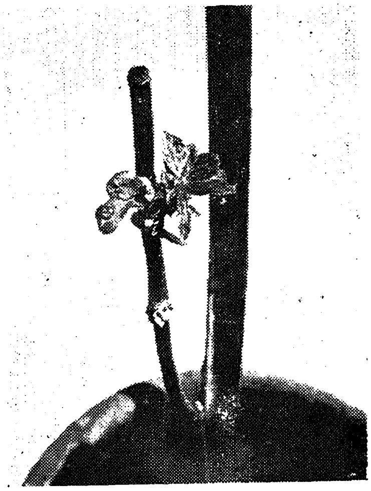

FIG. 2. Symptoms of passion flower mosaic on *Passiflora edulis f. flavicarpa* buddling.

Besides mechanical and graft transmission this disease was also transmitted by an aphid vector. *Aphis gossypii*, Glover was found naturally colonizing on *P. caerulea* vines. Apterus forms of *A. gossypii* from naturally infected plants when transferred to seedlings of *P. caerulea* grown from healthy cuttings in glass-house, transmitted the disease readily.

The mosaic disease under study resembles Passiflora mosaic virus reported from Malaysia by Ong Ching Ang and Tig Wen Ponh[3]: Passiflora ring spots reported from Ivory coast by De Wijs[2] and Passiflora virus diseases reported from Kenya by Bakker[1] in symptomatology and transmission by aphid vector and grafting. However, it differs from passion flower yellow vien mosaic virus disease reported from India by Wilson and Satyarajan[4] in symptomatology and transmission properties. The reaction of *C. amaranticolor* and *G. globosa* to the present virus is similar to passion flower mosaic from Malaysia. On the basis of symptomatology on *P. edulis f. flavicarpa* and transmission studies the present virus disease has been labelled as Passiflora mosaic virus similar to one reported from Malaysia. Further studies on the identification of this virus by serelogy and electron microscopy is in progress.

The occurrence of passion flower mosiac virus is recorded for the first time in India.

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---

1. Bakker, W., *E. African Agril. J.*, 1974, **40** (1), 11.
2. De Wijs, J. J., *Ann. Appl. Biol.*, 1974, 77, 33.
3. Ong Ching Ang and Ting Wen Poh., *MARDI Res. Bull.*, 1973, 1 (1), 33.
Wilson, K. I. and Satyarajan, P. K., *Sci. and Cult.*, 1970, **36** (4), 224.

POLYHAPLOIDY IN MAIZE (*ZEA MAYS* L.)

POLYHAPLOIDS are haploids originating from polyploids and Kimber and Riley[2] listed 21 genera and 37 species where polyhaploids have been observed by various observers. In addition to their list, polyhaploidy has also been reported in *Zea mays*[5], *Lilium longiflorum*[1], and *Sorghum almum* Parodi[4]. Significance of polyhaploidy as an evolutionary mechanism has been repeatedly emphasized by Kimber and Riley[2], Magoon and Khanna[3], Raven and Thompson[6], and Randolph and Fischer[5].

The occurrence of polyhaploids in angiosperms was first reported in maize by Randloph and Fischer[5] who pointed out the significance of polyhaploidy from the evolutionary standpoint in that the reduced diploids are capable of establishing new forms. It would, however, appear strange that no further study to elaborate the mechanism of polyhaploidy in maize has been reported so far.

In a previous study one definite case of polyhaploidy from tetraploid maize was recorded in a *pr*, 4*n* × multiply marked 2*n* crosses[7]. In subsequent studies we observed that freshly prepared tetraploid stocks produce parthenogenetic diploids much more frequently than the established tetraploid lines. To test this possibility we included a well established tetraploid line, Synthetic B 4*n*, a freshly developed tetraploid line obtained from 2*n*-Syn. B 4*n* crosses[8], and a tetraploid line selfed 10 generations after its synthesis, for comparison of polyhaploid-yielding frequencies. The seedling marker, *lg* (liguleless seedling), was available in all the lines and was used to isolate putative polyhaploids in *lg*, 4*n* × *Lg*, 4*n* crosses.

Porgeny from these crosses were raised in the field and the liguleless plants were selected as possible polyhaploid cases. They were crossed to diploid and tetraploid tester lines to confirm the ploidy level. The polyhaploids would produce plump kernels whe crossed to diploid ears and shrivelled kernels wh crossed to tetraploids. Pollen from liguleless tetraploid plants (originating from self-contamination), o the other hand, would produce shrivelled seeds in cross to diploids and plump seeds in tetraploid testers. Th results obtained from these studies are presented i Table I. Synthetic B tetraploid failed to show a singl case of polyhaploidy in 4,830 fertilizations, when the fresh tetraploid line produced 7 cases in 4,2 fertilizations giving a frequency of about 1 per 6 The other line which had undergone ten generatio of selfing after its synthesis showed a frequency about 1: 900.

TABLE I

Polyhaploid frequencies in lg, 4n ×Lg, 4n *crosses involving different females*

Female line	No. of fertilizations,	No. of liguleless plants	No. of poly-haploid
Synthetic B tetraploid	4,830	2	0
4*n* line selfed ten generations	2,745	4	3
Freshly synthesised 4*n* line	4,216	9	7

These results clearly establish that the freshly de loped tetraploid lines are more unstable and produ more parthonogenetic diploids than the older lin which had been stablised over the years. Diploidi tion of tetraploid lines in maize may be expected due to anomalies in sporogenesis and this phen menon can be gainfully utilized to study the mech nism of polyhaploidy in maize.

The cytological behaviour of the polyhaploid pla was quite regular with 10 bivalents appearing in the cells studied from 3 plants.

Seed set in the polyhaploid plants was normal w all the ovules developing. No cytological abnormal could be detected in the progeny of the polyhaplo

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1. Emsweller, S. L. and Uhring, J., *Amer. J. B* 1962, 49, 978.
2. Kimber, G. and Riley, R., *Bot. Rev.*, 1963, **29**, 4
3. Magoon, M. L. and Khanna, K. R., *Caryologi* 1963, 16, 191.
4. — and Shambhulingappa, K. G., *Genet. Pol* 1962, 3, 301.
5. Randolph, L. R. and Fischer, H. E., *Proc. N Acad. Sci.*, 1939, **25**, 161.
6. Raven, P. H. and Thompson, H. J., *Amer. N* 1964, **98**, 251.
7. Sarkar, K. R., *Diss. Abst.*, 1966, **27**, 1736B.
8. —, and Coe, E. H., Jr., *Crop. Sci.*, 1971, **11**, 5

IDENTIFICATION OF A MALE-STERILE GENE IN SORGHUM

THE development of random mating populations in sorghum was made possible with the availability of genetic and cytoplasmic-genetic male-sterility systems. So far, ten genetic male-steriles have been reported[1] but only a few of them are free from undesirable character associations. An efficient and easily identifiable male-sterility source is very important for effective random mating and recurrent selection.

This note describes a genetic male-sterility system in sorghum and reports its inheritance.

MATERIALS AND METHODS

Three male-sterile plants were observed in the sorghum line IS 104 in 1974 *rabi* season. The anthers were very small, thin, white and rudimentary (Fig. 1) and there were no traces of pollen in them. There was no female-sterility. This sorghum line had several desirable characteristics such as short stature, early maturity, bold and white grains (but with persistent sub-coat). No differences were noticed between male-sterile and fertile plants.

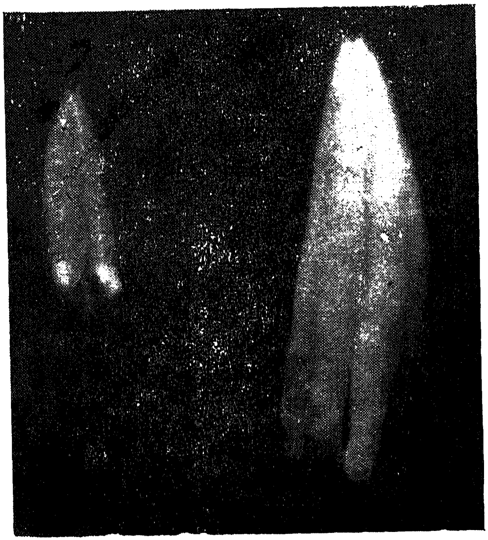

FIG. 1. Male-sterile (left) and fertile anthers.

One male-sterile plant was crossed with bulk pollen from all the fertile plants in the family. Seventy-five plants were grown in F_1 in 1975 summer. In 1975 *kharif*, an F_2 population of 688 plants was grown and male-sterile plants were identified at bloom. Fifty male-sterile heads were crossed with pollen from separate fertile plants (plant-to-plant sibbing). Ten F_2 fertile plants were grown as F_3 families along with 50 sibs in 1975 *rabi*. In each segregating family, steriles and fertiles were counted at flowering.

RESULTS AND DISCUSSION

All F_1 plants were fertile indicating that the male-sterility is not due to cytoplasmic factors but genetic in nature. The results obtained from F_2, F_3 and sibs are presented in Table 1. In the F_2 generation 171 male-steriles appeared out of a total of 688 plants which exactly fit into 3 : 1 ratio. The ten F_3 families derived from individual F_2 fertile plants had six families (60%) segregating in 3 : 1 ratio as against expected value of 6·67 (66·67%) on the basis that the ratio of fertile homozygous plants to the heterozygous fertile plants in F_2 is 1 : 2. These results are confirmed by the segregation pattern observed in families derived from plant-to-plant sibbing. Out of 50 families 35 (70%) segregated into 1 : 1 ratio and 15 (30%) families did not segregate. These results clearly show that the male-sterility reported here is inherited as a single gene recessive.

TABLE I

Inheritance of male-sterility in sorghum

Segregation in F_2	X^2	F_3 families	X^2	Sibs	X^2
517 (516)* F	0·006	6 (6·67) Sg	0·201	35 (33·33) Sg	0·250
171 (172) S		4 (3·33) NSg		15 (16·67) NSg	

* Figures in parentheses are expected values.
F = Fertile; S = Sterile;
Sg = Showing segregation;
NSg = Not showing segregation.

Morphological features of this male-sterility appear to be very distinct and superior to ms_3 and ms_7 genes, and it is much more easily recognised in the field. Comparatively the anthers are very small, thin and show complete sterility as against ms_3 gene which is often showing only partial sterility now. Genetic studies are in progress to determine whether it is different from them. A gene symbol will be proposed after confirmation. The stability of this gene in a wide range of genetic and cytoplasmic backgrounds is being tested.

The author is thankful to Drs. Hugh Doggett and Peter Lawrence for their comments and suggestions.

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The responsibility for all aspects of this publication rests with the International Crops Research Institute for the Semi-Arid Tropics.

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1. Ross, W. M., Grain Sorghum Producers Assn., Grain Sorghum Research and Utiliszation Conference Seventh Beinnial Programme March 2–4, 1971, Lubbock, Texas, p. 93

PHAEOISARIA HOHNEL, A NEW GENERIC RECORD TO INDIAN HYPHOMYCETES

IN the course of a mycological survey of the forests of Kerala during January 1976, an interesting hyphomycetous fungus was collected on two different hosts, from two different localities. On critical examination and study, the two collections were found to agree in all respects with *Phaeoisaria clematidis* (Fuckel) Hughes. Since the form-genus *Phaeoisaria* Hohnel reported herein, constitutes a new generic record to India and being of rare occurrence, a brief description of the same, along with an illustration, is presented here for ready reference.

Phaeoisaria clematidis (Fuckel) Hughes (Fig. 1)

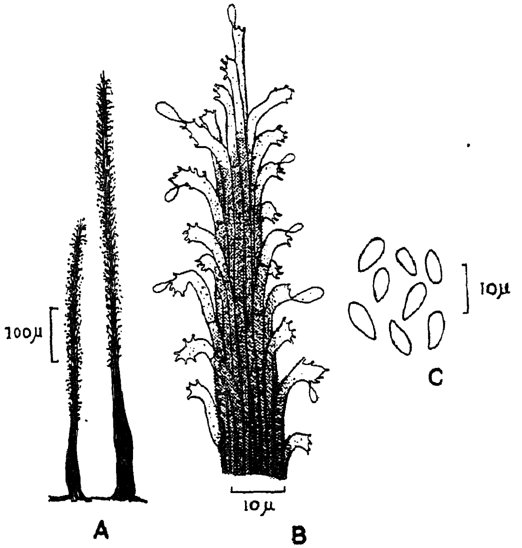

FIG. 1. *Phaeoisaria clematidis*: A. Synnemata, B. Top of the synnemata showing conidial attachment, C. Conidia.

Mycelium partly superficial and partly immersed. Synnemata black, hairy, upto 1·25 mm high; 15–35 μ thick at the base, 7·5–15 μ thick at the apex, individual threads narrow, branched towards their apices, splaying out at the apex and along the sides of each synnema, pale to mid-brown and 2–3 μ thick. Conidiogenous cells cylindrical hyaline to pale brown with numerous cylindrical denticles. Conidia solitary, dry, fusiform; narrowly ellipsoidal, hyaline, smooth; 1-celled; measure 5–8 × 2–3·5 μ.

Matrix:

(*i*) On dead twings of *Aegle marmelos* Corr. Legit. K. I. M. V. (23–1–1976) Moonnar, Kerala, No. AMH 3046.

(*ii*) On dead stems of a palm. Legit. K. I. M. V. (20–1–1976) Waynaad, Kerala, No. AMH 3047.

The writers are grateful to Prof. M. N. Kam for his keen interest, to the Director for laborato facilities and to the Ministry of Education, Govt of India, for the award of S.R.T. to one of u (K. I. M. V.).

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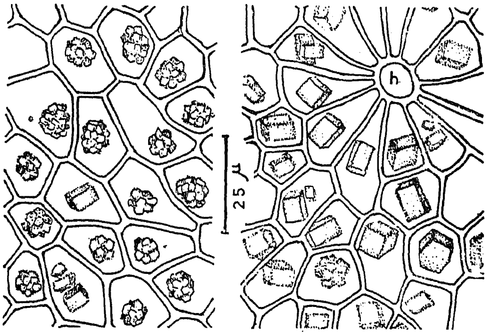
---

1. Ellis, M. B., *Dematiaceous Hyphomycetes*, C.M.I., Kew, England, 1971, p 608.
2. Mukerji, K. G. and Juneja, R. C., *Fungi of India* (*1962–1972*), Emkey Publ., Delhi, 1975, p. 224.

A SIMPLE MORPHOLOGICAL TECHNIQUE FOR DISTINGUISHING THE SEX OF NUTMEG SEEDLINGS

NUTMEG (*Myristica fragrans* Houtt.) is an impo tant spice plant of the world, but its popularity a a plantation crop is seriously handicapped by it dioecious nature and prolonged preflowering period. In any plantation of nutmeg, 40–45% of plant turn out to be male and nearly 5% bisexual[1-] Only the female plants are of commercial value. Phadnis and Choudhari[3] recently reported som color difference between the leaf extracts of mal and female nutmeg plants when treated wit ammonium molybdate but this method is rathe cumbersome. A study of the foliar epidermis o a large number of nutmeg plants cultivated on th West Coast of South India revealed that calciu oxalate crystals found in the epidermal cells ar different in form in the male and female plant and this can be employed as a method of sexin the plants five to seven years before the plan come to flower.

The epidermal cells are sparsely chlorophyllo polygonal, 15–40 μ across, having straight later walls. Very young leaves bear uniseriate deciduo hairs of characteristic shape scattered along t lower epidermis. In mature leaves the epiderm cell bearing the hair remains small and circular outline; the surrounding cells are radially elongat Nearly all cells contain globose hyaline to p yellowish-green oil globules. Several small (2– across) crystals of calcium oxalate are found each cell in young leaves of both male and fem plants. A single large rhomboidal or prism crystal with rectangular or squarish flat faces occ in the epidermal cells of mature leaves (usually 5th leaf from the apex of branch and those bel of the male plant (Fig. 2); one or two small crystals of the type found in the young leaves are seen in addition in some cells. In the epidermal cells of female plants (Fig. 1) the crystals are clustered to form large compound spherical masses (druses). Some epidermal cells of the male plants may have compound crystals of the female type; simple prismatic crystals of the male type are occasionally found in some epidermal cells of the female plant. The bisexual trees are similar to the male trees in the nature of the crystals. A study of 4-year-old grafted plants, in which seedlings of *M. contorta* Warb. are used as stalk, reveals that there is no apparent difference in the nature of the crystals in the foliar epidermis, even though crystals are abscent in foliar epidermis of *M. contorta.*



1 2

FIGS. 1–2. Fig. 1. Portion of lower foliar epidermis of an adult plant of *M. fragrans.* Fig. 2. Same, of a male plant (*h*—hair-bearing cell.)

As in the young leaves of adult plants, the foliar epidermis of young seedlings (one year old) contain 2–10 small prismatic crystals of nearly equal size. One of these crystals grows conspicuously larger as seedlings grow and gradually all or nearly all the others wane off in some plants while in the others the crystals get aggregated towards the middle of the cell. By the time the seedlings are two years old it is possible to distinguish the two types of plants, one having simple crystals and the other druses, even though the distinction is not as marked as in the adult plants. An analysis of 80 plants three to four years old in the University orchard revealed that 41 of them possess simple crystals of the male type and 39 possess druses in the cells of their lower foliar epidermis. This proportion of 51 : 49 nearly corresponds to the reported 50 : 50 proportion between male (including bisexual) and female plants in seed-raised plantations. It is reasonable to state that the sex of nutmeg plants can be identified from the nature of the crystals found in the lower foliar epidermis of seedlings which are two or more years old *i.e.,* five to seven years before the seedlings come to flower.

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1. Anonymous, *The Wealth of India* (Council Sci. Industr. Res., New Delhi), 1962.
2. Dienem, K., *Landbouw, Buitenz,* 1931, 1, 647.
3. Phadnis, N. A. and Choudhary, K. G., *Trop. Sci.,* 1971, 13, 265.

HERBICIDAL POLLUTION-CHLOROPHYLL CONTENT AS AN INDEX OF RESIDUAL TOXICITY

PREFIX [2, 6-dichlorothiobenzamide], 2, 4-D [2, 4-dichlorophenoxyacetic acid], Bladex [2-(4-chloro-6-ethylamino-S-triazin-2-ylamino)-2-methyl propionitrile] and Planavin [4-(methyl sulphonyl)-2, 6-dinitro-N, N-dipropyl aniline] were mixed in the garden soil on 50 ppm by weight basis. The treated soils were kept in pots. About 20 cm rainfall was recorded during the exepriment. Additionally these soils were leached with 16 litres of water. The gram (*Cicer arietinum*) was grown in these pots as test plant. Leaves of one month old plants were sampled for chlorophyll content and it was estimated by Bray[2] (1960) formula.

Apparently no difference could be marked in the leaves of the test plant grown in treated soils. But the data (Table I) indicate a reduction in chlorophyll contents with all the types of herbicides belonging to the different chemical groups. Planavin, residues appeared most toxic as about 30·2% reduction was recorded. This is followed by Bladex (21·6%), 2, 4–D (13·11%) and Prefix (4·5%). It is known that different groups of herbicides have different modes of action as Prefix inhibits the respiration and protein synthesis

TABLE I

Chlorophyll content in the gram grown in treated soils

Herbicide	Chlorophyll content mg/g fresh weight				
	a	*b*	*a/b* Ratio	Total	% Reduction
Control	2·004	1·329	1·5	3·333	..
Prefix	2·242	0·946	2·3	3·188	4·5
2, 4–D	2·164	0·734	2·9	2·898	13·11
Bladex	1·872	0·736	2·5	2·608	21·6
Planavin	1·580	0·730	2·1	2·310	30·2

(Smith *et al.*, 1971[13]; Ashton and Crafts, 1973[1]), phenoxys (2, 4–D) inhibits growth and Rnase activity (Ashton and Crafts, 1973[1], Corbett, 1974[4]), and nitroanilines (Planavin) increase the carbohydrate content but affect nucleic acid metabolism and root growth (Schieferstein and Hughes, 1966[12]; Ashton and Crafts 1973[1]). But in the present studies all the chemicals inhibited the biosynthesis of chlorophyll. The case of Bladex supports Glabiszewski *et al.* (1966)[10] and Gorden and Monselise (1967)[11] that it damages chlorophyll. The side effect of herbicides (Dubey and Mall, 1972[6]; Dubey, 1974[7]) and fate of pesticides has been critically reviewed (Crosby, 1973[5]; Burnside, 1974[3]) indicating their disappearance in the environment but the residual assessment of the herbicides used confirms that these compounds are slow in getting leached, and are least bio, thermo and photo degradable (Dubey and Rao 1974[8], 1975[9]) and hence express strong residual activity. This quantitative reduction in chlorophyll indicates the pollution potentials of these herbicides with reference to the soils of this region.

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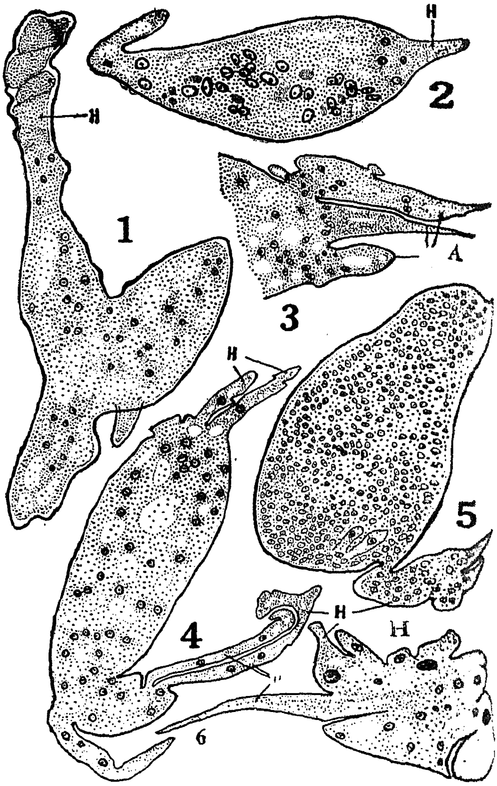
1. Ashton, F. M. and Crafts, A. S., *Mode of Action of Herbicides*, John Wiley and Sons, Inc., N.Y., 1973.
2. Bray, J. R., *Can. J. Bot.*, 1960, **38**, 313.
3. Burnside, O. C., '*Pesticides in Soil and Water*, Soil Sci. Soc. of America, 1974, p. 387.
4. Corbett, J. R., *The Biochemical Mode of Action of Pesticides*, Academic Press, London, 1974.
5. Crosby, D. G., *Ann. Rev. Pl. Physiol.*, 1973, **24**, 667.
6. Dubey, P. S. and Mall, L. P., *Experientia*, 1972, **28**, 600.
7. —, *Geobios*. 1974, **1** (6), 178.
8. — and —, Rao, A. H., *Curr. Sci.*, 1974, **44**, 247.
9. — and —, Paper in *IInd Photobiology Symposium*, Nainital, India, 1975 (28–30 April).
10. Glabiszewski, J. M., Plonynski, M., Szumilak, G. and Zurawski, H., *Pam Pulaw*, 1966, **21**, 233.
11. Gorden, R. and Monselise, S. P., *Plant Physiol.*, 1967, Abstr. **40**, XV.
12. Schieferstein, R. H. and Hughes, W. J., *Proc. 8th Br. Weed Cont. Conf.*, 1966, p. 377.
13. Smith, L. W., Peterson, R. L. and Horton, R. F., *Weed. Sci.*, 1971, 19, 174.

HAUSTORIAL APPENDAGES OF GIANT CELLS: ROOT-KNOT OF TOMATO (*MELOIDOGYNE INCOGNITA* CHITWOOD)

THE infective larva of the root-knot nematode enters the root and embeds its anterior end in the stele of the root. Profound histological changes are induced by inciting hypertrophy and hyperplasia in the pericycle, and xylem parenchyma of the host root. However, the nematode does not feed indiscriminately on these tissues. It incites in the soft stelar tissues around its head, a number of densely protoplasmic and multinucleate giant cells on which the nematode feeds.

The giant cells are formed either by repeated free nuclear mitotic divisions, without cell wall formation in a uninucleate hypertrophied initial cell[1] or a number of uninucleate initial cells fuse together by dissolution of their adjacent walls forming a syncytium in which the nuclei may divide mitotically[2,3] or before fusing, the uninucleate initials become multinucleate and later the nuclei may divide mitotically[4].

Many nematologists have studied the structure of giant cells by means of anatomical techniques including macerations and even by the electron microscope[5]. However, none so far has reported the appendages of the giant cells, described here (Figs. 1–6).



FIGS. 1–6. *Meloidogyne incognita*, giant cells wi haustorial appendages (H), × 300. Fig. 3. portion of the giant cell with pointed appendage (A. appendages.)

It was suspected that the giant cells poss some mechanism for absorbing nutrients from adjacent stelar parenchyma. Therefore, the galls were cut into bits and macerated. The giant cells were dissected out. It was observed that they bear various kinds of irregular haustorial appendages, often with pointed ends (Figs. 3, 6). The appendages are intercellular and so densely protoplasmic that with haematoxylin, the nuclei and protoplasm take such a deep stain that the nuclei are hardly visible (Figs. 1, 2).

Some giant cells contain a large number of nuclei (Fig. 5) but others have a few of them (Figs. 2, 6). A giant cell shows some nuclei larger than others (Fig. 6).

We are thankful to the Head of the Department of Botany, University of Rajasthan, for facilities.

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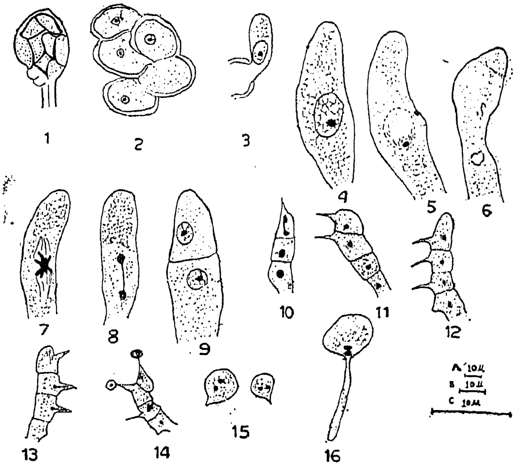
---

1. Huang, C. S. and Maggenti, A. R., *Phytopath.*, 1969, 59, 447.
2. Christie, J. R., *Ibid.*, 1936, 26, 1.
3. Dropkin, V. R. and Nelson, P. E., *Ibid.*, 1960, 50, 442.
4. Owens, R. G. and Specht Helen, N., *Contrib. Boyce Thompson Insti.*, 1964, 22, 471.
5. Paulson, R. E. and Webster, J. M., *Canad. J. Bot.*, 1970, 48, 271.

CYTOLOGICAL STUDIES IN THE GERMINATING TELIOSPORES OF *RAVENELIA ESCULENTA*

DETAILED observations on the behaviour of the diploid nucleus in the germinating teliospores of *Ravenelia esculenta* Naras. and Thirum., an autoecious rust, which parasitizes *Acacia eburnea* Willd. have been made. The telial material was collected near Nipani (Karnataka State) and the teliospores were germinated by hanging drop method. The spores germinated within 5 to 7 hours at room temperature without any resting period and remained viable for more than 10 days. Germinating teliospores were fixed at different stages of development in Allen's modified Bouin's fixative and stained in Heidenhain's haematoxylin and counterstained with Orange G. 1% acetocarmine stain also yielded satisfactory results.

The teliospore head consists of 4 to 12 spores grouped irregularly. The pedicel is compound and composed of 2 to 3 hyphae. There is a pendent cyst at the base (Fig. 1). In most of the cases more than 3 spores germinated from the spore head. Under excess moisture conditions, prolongation of germ tubes and sterigmata occurred. Each teliospore cell contains one diploid nucleus (Fig. 2). The teliospore cell germinates by giving rise to a papilla which elongates rapidly. The diploid nucleus migrates into the promycelium (Fig. 3). The migrating nucleus has a clear nuclear membrane with chromatin reticulum. Nuclear division commences when the interphasic nucleus approaches the middle part of the badidium (Fig. 4). At a later stage the nuclear membrane starts disappearing while the nucleolus and splitting reticulum move towards the periphery (Fig. 5). In the late prophase I the chromosomes spread out into two groups forming Diakinesis (Fig. 6).



FIGS. 1–16. Fig. 1. Teliospore head with pedicel, cyst and irregularly arranged spores. Fig. 2. Mature teliospore with diploid nucleus. Fig. 3. Migrating nucleus. Fig. 4. Nucleus in the middle of the Basidium. Fig. 5. Disappearing nuclear membrane with peripheral nucleolus and reticulum. Fig. 6. Diakinesis. Fig. 7. Metaphase I. Fig. 8. Anaphase I. Fig. 9. Late telophase I. Fig. 10. Division II, Anaphase II, upper nucleus. Figs. 11 and 12. Formation of sterigmata. Fig. 13. Migration of nuclei in the sterigmata. Fig. 14. Basidiospores on the sterigmata. Fig. 15. Binucleate basidiospores. Fig. 16. Germinating basidiospores.

(Magnifications : A—Fig. 1, B—2, 3, and 10 to 14, C—4–9 and 15 & 16).

During metaphase I, the chromosomes are at their shortest length. In the metaphase plate the chromosomes are arranged very compactly. Orientation of the metaphase spindle is rather indistinct with ill-defined fibres (Fig. 7). In anaphase I, the spindle contracts and the fibres coalesce into a single strand (Fig. 8). In telophase I the daughter nuclei are puite prominent and are separated by first cross wall formation (Fig. 9). A resting phase does not seem to occur as the first meiotic division is immediatly followed by a second division (Fig. 10). The nuclear behaviour in the second division is of a short duration as it is an equational one.

Even before the completion of the second nuclear division sterigmata are formed (Figs. 11, 12) which, in turn, bear the terminal basidiospores (Fig. 14). The nuclei and much of the cytoplasm migrate into the developing basidiospores through sterigmata (Fig. 13). The young basidiospore is uninucleate (Fig. 14) while the mature one contains two nuclei as a result of third nuclear division (Fig. 15). The basidiospores germinate sooner or later after detachment from the sterigmata.

In this rust the fusion nucleus undergoes three successive divisions instead of two (which is normal for most rust fungi) the first two occurring in the basidium followed by the third in the basidiospore. Allen[1] and Ashworth[2] have independently reported similar results with *Puccinia malvacearum* Burt.

Grateful thanks are conveyed to Dr. G. V. Joshi, for laboratory facilities.

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---

1. Allen, R. F., *Phytopath.*, 1933, 23, 572.
2. Ashworth, D., *Trans. Brit. mycol. Soc.*, 1931, 16, 177.

OCCURRENCE OF E-TYPE BRIDGES IN *ALOE BARBADENSIS* MILL.

Aloe barbadensis Mill. (*A. perfoliata* var. *vera* L., *A. vera Auth.* non Mill.) is known for its medicinal properties. While working out the details of its meiosis, E-type bridges have been recorded in a few pollen mother cells (Fig. 1). The bridge is formed during anaphase-I and assumes the appearance of a capital E, hence the name E-type.

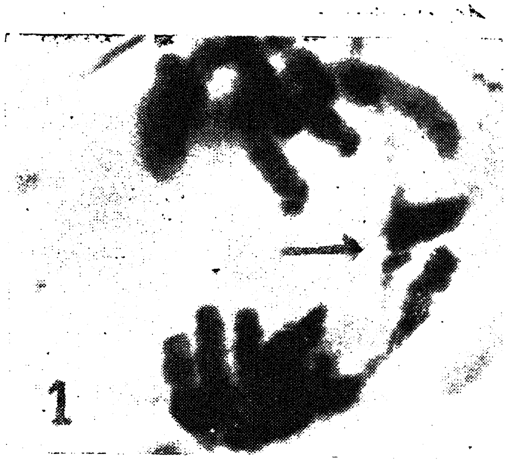

FIG. 1. Late anaphase showing formation of E-type bridge, × 1,650.

Occurrence of E-type bridges, a little known meiotic event, has been reported earlier[1-3], but it was Brandham[4] who, for the first time, logically explained the formation of E-type bridges in the tribe Aloineae of Liliaceae. Bivalents involved in the formation of such bridges are heterocorphic with regard to the relative lengths of the long and the short arms. A pericentric inversion followed by a paracentric inversion results in the formation of heteromorphic homologues. Single crossover involving a segment in the long arm of normal chromosome pairing, with its homologous segment situated in the short arm of the inverted homologue, results in the formation of an E-type bridge and a large acentric fragment in anaphase-I; the latter may get lost sooner or later. High incidence of such bridges suggests interspecilfi hybridity.

Grateful thanks are extended to Prof. K. B. Deshpande for facilities, to Dr. P. E. Brandham, Royal Botanic Gardens, Kew, England, for confirming chromosomal configurations, and to U.G.C. for partly financing the scheme—'Cytological Studies in the Tribe Aloineae'.

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---

1. Ribbands, C. R., *J. Genet.*, 1937, 1, 35.
2. Sato, D., *Jap. J. Bot.*, 1942, 12, 132.
3. Darlington, C. D. and Keffallinou, M., *Chromosoma*, 1957, 8, 364.
4. Brandham, P. E., *Ibid.*, 1969, 27, 201.

NEW CYTOPLASMIC MALE STERILE LINES OF SUNFLOWER

SUNFLOWER hybrids in comparison with the ope
pollinated varieties were reported to exceed standar
varieties by about 54% in seed yield[3] and 34% in o
content[8] in addition to being more uniform wi
regard to maturity, plant height and vigour. Cons
quently a number of hybrids using self-incompatib
lity and male-sterility were produced and are in cul
vation in France, Rumania, U.S.A. and Canada[2,4]-

As self-incompatibility in sunflower is not comple
in the production of hybrid using this mechanis
selfed seeds upto 35% have been recorded in S_3–
inbred lines used as female parents[7]. Thus the f
use of heterosis cannot be made. On the other han
due to inbreeding, the production of highly se
incompatible lines and their maintenance would
difficult. The genetic male sterility due to a recess
gene linked with green colouring in seedlings requi
seeding at high rates and increased cost of labour
the removal of anthocyanic male fertile plants.
was also found that genetic male sterility could

TABLE I

Agronomic characters of male sterile lines of sunflower (Rabi 1974–75)

Male sterile lines	Characters							
	Days to 75% flowering	Days to 75% maturity	Plant height (cm)	Head diameter (cm)	Seed filling (%)	Yield/plant (g)	Husk (%)	Oil (%)
Pcms–1	106	134	161	14	78	40	27	41
Pcms–2	98	126	134	14	41	38	28	40
Pcms–3	96	130	137	13	86	41	31	43
Pcms–4	96	130	146	14	70	41	28	41
Pcms–5	113	149	135	13	85	45	30	45

altered by the presence of modifier genes and the linkage was not complete[8], thereby necessitating roguing in both male and female rows. Finally the discovery of cytoplasmic male sterility in *Helianthus petiolaris* by Leclercq[8] provided most economic and efficient source of hybrid seed production in sunflower and using this material hybrids have been developed in France, Rumania and U.S.A.[4–5].

Twenty-two male sterile plants were observed in "1970 R Composite" obtained from Dr. M. L. Kinman of U.S.D.A., College Station, Texas, in 1972. Paired crosses with male fertile plants produced progenies with varying degrees of pollen fertility restoration. Male sterile plants from F_1 progenies were crossed with 79 accessions. As the occurrence of pollen fertility restorer genes is reported to be quite common in sunflower, only 5 varieties, *viz.*, C.P.I. 14990 (Australia), Ireqiekos (Hungary), Commander (Canada), Armavirskiij 3497 and Smena (U.S.S.R.) which did not restore any pollen fertility could be detected. Through 4 successive back crosses, 5 male sterile lines were developed whose agronomic characters are given in Table I.

More than 600 S_8–S_4 inbreds have been crossed with the above male sterile lines. As varying degrees of pollen fertility restorations were observed in crosses with accessions from which the above inbred lines were developed, it is expected that a large number of hybrids with at least partial fertility restoration will be obtained. The hybrids with only 25% pollen fertility restoration could be cultivated commercially. Moreover, complete restorers and non-restorers with high selfing are also expected which will be of immense value in hybrid sunflower production.

Authors are grateful to Dr. Maharaj Singh, Director, Experimental Station, for constant encouragement and facilities.

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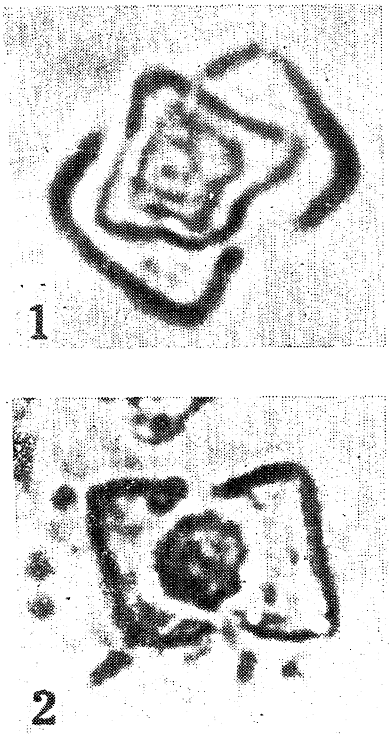
1. Enns, Henery, Dorrel, D. G., Hoes, J. A. and Chubb, W. O., *Proceedings of the Fourth International Sunflower Conference*, 1970, p. 162.
2. Gimenez, J. D. and Fick, G. N., *Crop Sci.*, 1975, **15**, 725.
3. Grebenjuk, G. K., *Ser. Brd. Agric. Sci.*, 1967, **11**, 51,
4. Kinman, M. L., *Proceedings of the Fourth International Sunflower Conference*, 1970, p. 181.
5. Leclercq, P., *Ibid.*, 1970, p. 123.
6. Singh, D., *Sunflower-Industry in U.S.S.R.*, Department of Agriculture, Lucknow, U.P., 1972.
7. Vranceanu, V., *cf. Pl. Br. Abstt.*, 1967, p. 3176.
8. —, *Proceedings of the Fourth International Sunflower Conference*, 1970, p. 136.

OBSERVATIONS ON ASEXUAL REPRODUCTION IN *TETRAEDRON* KÜTZ.

As known so far, asexual reproductitn in *Tetraedron* Kütz. takes place by the formation of several autospores by successive divisions of the cell contents[2-4], although zoospore formation has been reported in *T. bitridens*[4]. The pyrenoid does not divide but is transferred to one of the daughter masses and ultimately degenerates: each autospore developing its own pyrenoid in due course[2,3].

During observations on *Tetraedron minimum* (A. Braun) Hansgirg[1] var. *scrobiculatum* (Lagerheim) De Toni[4], the authors observed variant stages of asexual reproduction.

In the present material collected from a tank in Lucknow University campus, it was observed that generally, only a single autospore was formed in each cell, although rarely, 2–4 autospores were also noticed. The cytoplasm of the parent cell contracts and develops a wall assuming identical features as the mother cell, before it is liberated by the rupture of the mother cell wall along the two deeply concave sides (Fig. 1). The pyrenoid in such cases persists and never degenerates and is observable throughout the development of the autospore up to its maturity. In cases where the contents divide into 2–4, some daughter protoplasts mature into autospores while 1, 2 and 3 or them gradually degenerate.



Figs. 1–2. *Tetraedron minimum* var. *scrobiculatum*. Fig. 1. Showing single autospore formation and its liberation along two deeply concave sides, (× 2,500). Fig. 2. Formation of a single aplanospore and rupture of the parent cell wall, (× 2,000).

Sometimes, spores with a very thin outer membrane and devoid of flagella, (aplanospores) are formed singly (Fig. 2) or 2, 4 or 8 within parent cells. They are liberated in the same manner as autospores and attain the normal thick wall gradually outside the parent cell. Formation of single autospore and aplanospores and the persistence of the pyrenoid from parent cell to the daughter cell are features hitherto unrecorded for the genus *Tetraedron*[4].

The present material slightly differs from *Tetraedron minimum* var. *scrobiculatum* in possessing a prominent papilla at each angle and a minutely wavy cell wall.

Two of us (Y.S. & R.K.M.) thank C.S.I.R. and U.G.C., respectively for financial assistance during the tenure of the present work.

Department of Botany,
Lucknow University,
Lucknow,
July 27, 1976.

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Yashpal Singh.
R. K. Mehrotra.

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1. Brühl, P. and Biswas, K., "The algae of Bengal filter beds," *J. Dep. Sci., Calcutta Univ.*, 1922, 4, 1.
2. Fritsch, F. E., *Structure and Reproduction of the Algae*, Vol. I, Cambridge, 1935, pp. 791.
*3. Geitler, L., "Die Entwicklungsgeschichte von *Sorastrun spinulosum* and the Phylogenie der Protococales. *Arch. Protistenk*, 1924, 47, 440.
4. Phillipose, M. T., *Chlorococcales.*, I.C.A.R. Pub., New Delhi, 1967, pp. 365.

* Not seen in original.

A NEW FRUIT ROT OF *PSIDIUM GUAJAVA*

During a survey of the local fruit market, a hitherto unrecorded, severe, soft rot disease of guava (*Psidium guajava*) fruit was observed. The infected portion becomes macerated, soft, pulpy, water soaked, light brown in colour (Fig. 1). The complete internal rotting in 8–10 days was evident on cutting open the infected fruit.

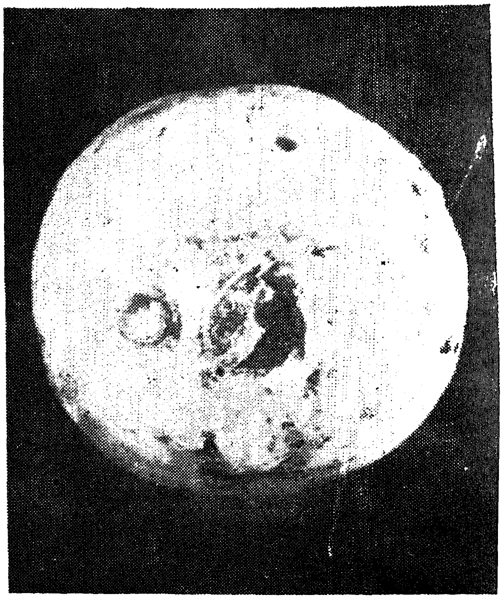

Fig. 1. Symptoms of *G. roseum* on Guava f

The causal organisms was isolated from diseased fruit of guava and identified as *Gliocladium roseum* Bainier. The fungus grew well on potato dextrose agar and host pulp agar media. Some healthy fruits of guava were inoculated with the fungus by inoculum disc after pricking the fruit surface and also by spraying. After seven days, the fruits pricked showed disease symptoms. From the inoculated fruit the same fungus was re-isolated.

The authors are grateful to Prof. S. B. Saksena, Head, Department of Botany, for providing facilities and also to the C.S.I.R., for the award of Junior Research Fellowship to senior author.

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PAECILOMYCES FARINOSUS (DICKSON ex FRIES) BROWN AND SMITH A NEW FUNGAL PARASITE OF THE MANGO LEAF WEBBER, *ORTHAGA EXVINACEA* H.

DURING February–March 1976, several larvae of the mango leaf webber, *Orthaga exvinacea* (Noctuidae : Lepidoptera) killed by *Paecilomyces farinosus* (Deuteromycetes : Moniliaceae) were collected from the Agricultural College Farm, Vellayani, Kerala. In India *P. farinosus* has been reported on the whitefly, *Bemisia tabaci*[1]. There is no previous record of its occurrence on *O. exvinacea.*

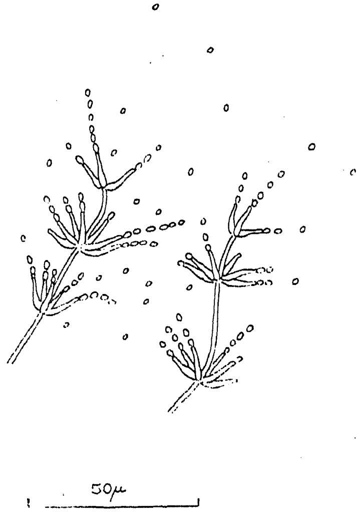

FIG. 1. *Paecilomyces farinosus.*

The fungal colony on potato-dextrose-agar medium has a tough matted basal felt and a loose hairy hyphal growth. The phialides (Fig. 1) are in whorls upto 7 and are roughly flask shaped. The conidia are hyaline, broadly elliptical and are found in short chains. The larvae inoculated by topical application of spores, exhibited loss of appetite, general sluggishness and decreased irritability within 48 hours. Towards the later stages, diseased larvae appeared lighter in colour than the healthy ones. Death occurred in 3 to 4 days. Under moist conditions whitish external growth of the fungus appeared 24 hours after death. The fungus was found to be highly pathogenic causing over 95 per cent mortality in the inoculated larvae.

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---

1. Nene, Y. L., *Indian J. Agric. Sci.*, 1973, 43, 514.

OBSERVATIONS ON THE POLLEN GRAINS OF *GYROCARPUS AMERICANUS* JACQ.

Gyrocarpus (Hernandiaceae ; Cronquist)[2] is a tropical genus represented in India by the species *G. americanus* Jacq. characterised by polygamous inflorescence, with numerous male, and a few female and bisexual flowers. The family has been variously treated by different taxonomists[1]. Pax[8] and also Cronquist[2] placed the family under Magnoliales, while Hutchinson[5] placed it in Laurales, which are currently considered to constitute the primitive Magnolian angiosperms.

In *Gyrocarpus jacquini* (*G. americanus* Jacq.) from Australia, Erdtman[4] observed non aperturate and spinulate pollen grains, with an average diameter of $45\,\mu$. Walker[9] made a mention of the pollen morphology of the family. Hardly any information is available on the different aspects of the biology of the taxa of Hernandiaceae as a whole.

The material for the present study was collected from the deep forest at "MEKE DHATU" near Kanakapura (Bangalore District) of Karnataka State, India, during February 1976. The material was preserved in glacial acetic acid and pollen preparations were made by the alcohol method of Nair[8] in which the protoplasm is not dissolved as compared with the acetolysis method.

The pollen grains are inaperturate and spinulate (very small), and are provided with very thin exine and sparse spinules on the exine surface (Fig. 1). The most notable feature in the present material is the large range of size variations from 30–64 μ (size classes: $30\,\mu = 4{\cdot}4\%$, $34\,\mu = 14{\cdot}8\%$, $40\,\mu = 14{\cdot}4\%$, $45\,\mu = 19\%$, $49\,\mu = 13\%$, $53\,\mu = 8\%$, $56\,\mu = 6{\cdot}6\%$, $60\,\mu = 3{\cdot}2\%$, $64\,\mu \pm 2\%$), based on measurements of a total number of 258 pollen grains (Fig. 2).

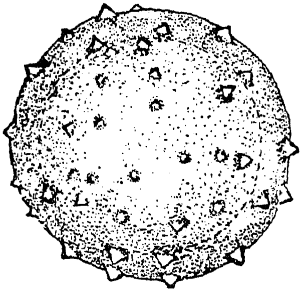

FIG. 1. Palynogram of *G. americanus* Jacq. showing the inaperturate and spinulate characteristics, × 400.

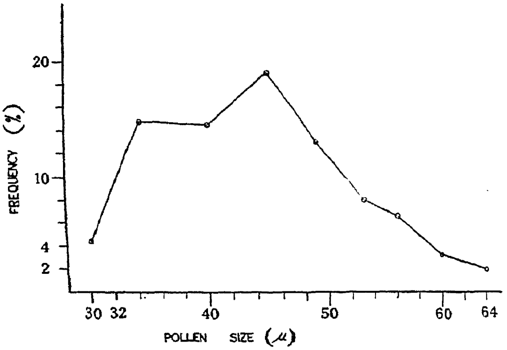

FIG. 2. Graph showing frequency of pollen size class in *G. americanus* Jacq.

The inaperturate pollen is indicative of the affinity of *Gyrocarpus* (Cyrocarpoideae) with Lauraceae. Erdtman[4] indicated that the pollen grains in Gyrocarpoideae are more or less similar to those in Lauraceae whereas the grains in Hernandioideae are slightly similar to those in *Peumus* (Monimiaceae). Guillaumin (Erdtman[4]) and Takhtajan[1] raised the Gyrocarpoideae of Engler and Prantl to the level of a family, Gyrocarpaceae. The palynological findings of Erdtman[4] and Walker[9] favour the separation of these two subfamilies. However, a final solution to this problem may be provided by embryological and other data of which hardly any knowledge is available[3].

The large range of size variations and the sparse distribution of spines are unique in the present material as compared with the observations made by Erdtman[4] in *G. jacquini*. Such variations in size and other morphological characters of pollen are considered to be an evidence of mutational changes in plants and therefore it may be presumed that the individual from which the present material has been procured (or the whole colony of them) constitute a bioform. The material having been collected from the deep forest, there is hardly any possibility of any biotic factor involved in inducing the genetic change. Therefore, it is possible that some unknown ecological or geographical factors have caused the change as reflected in pollen morphology.

The Magnoliidae of Cronquist, as a whole are characterized by the trimorphous apertural condition, there being trichotomololpate, monocolpate and inaperturate grains (Nair)[7]. In the scheme of pollen evolution, it has been generally considered that inaperturate condion is a speciallized feature, although Walker[9] and Muller[6] considered it to be a sign of a primitiveness as far as angiosperms are concerned. However, the flower structure of the Lauralas may be deemed to suggest higher evolution within the Magnoliidae (of Cronquist)[2] because of their apetalous condition. The modern thinking on angiosperm phylogeny is that the petaloid form like that of *Magnolia* is more primitive and the apetalous ones advanced.

Thanks are extended to Dr. P. K. K. Nair, Head, Polynology Laboratory, National Botanic Gardens, Lucknow, for his valuable guidance and advice; to Dr. T. N. Khoshoo, Director, National Botanic Gardens, Lucknow, for encouragement and to C.S.I.R. for providing Junior Fellowship to one of the authors (C. R. K.).

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September 28, 1976.

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1. Becker, K. M., *Taxon*, 1973, 22 (1), 19.
2. Cronquist, A., *Evolution and Classification o Flowering Plants.*, Houghtan Miffling Boston, 1968.
3. Davis, G. L., *Systematic Embryology of th Angiosperms*, 1960.
4. Erdtman, G., *Pollen Morphology and Pla Taxonomy—Angiosperms*, 1952, p. 202.
5. Hutchinson, J.. *The families of Flowerin Plants—Dicotyledons*, Vol. 1, 1959.
6. Muller, J., *Biol. Rev.*, 1970, 45; 417.
7. Nair, P. K. K., *Pollen Morphology of Angi sperms A Historical and Phylogenetic Stud*, Vikas Publishing House, Delhi, 1970.
8. Pax, F., In *Die Naturlichen Pflanzenfamilie* (Ed) Engler and Prantl, 1894, 3 (2), 129
9. Walker, J, W., "Evolutionary significanct of th exine on the pollen of primitive angiosperms in *the Evolutionary Significance of th Exine*, (Ed.) Ferguson, I. K. and Muller, Academic Press, London, 1976, p. 251.

ALTERNARIA MACROSPORA ZIMM. A NEW RECORD ON PASSION FRUIT (*PASSIFLORA EDULIS* SIMS.) FROM INDIA

DURING December 1975 a leaf spot was observed to be severe on purple variety of passion fruit at Horticultural Experiment Station, Chethalli. The disease symptoms were observed on the leaves and branches (Fig. 1). The disease appears on the leaves as small dark brown concentric spots which later enlarge and become irregular. In the later stages of the disease, yellowing of the leaves was also observed which resulted in premature drop. The disease symptoms on the branches are characterized by the production of dark brown lesions which result into the girdling effect and eventually death of the branches.

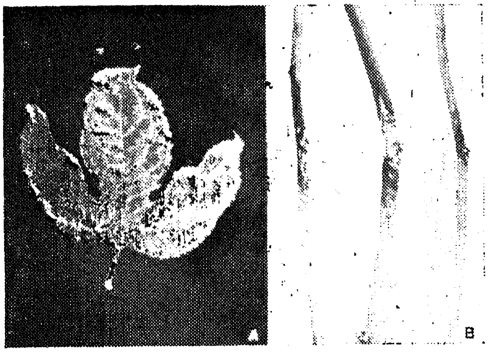

FIG. 1. Symptoms of *A. macrospora* on passion fruit, *a*. Leaf ; *b*. Branch.

The leaf spots and lesions on branches resulted in isolation of *A. macrospora*. The pathogenicity of the fungus wsa confirmed by spraying conidial suspension on two month old seedlings of purple passion fruit. The inoculated seedlings were kept in moisture chamber for 48 hours. Small flecks appeared on the fourth day. The larger dark brown lesions developed in 10 days and caused yellowing and defoliation of inoculated leaves in about two weeks. The branches were inoculated be syringe method using the conidial suspension. The characteristic lesion developed in 10–15 days resulting in girdling and finally death of the branches. Reisolation from the artificially inoculated plants yielded *A. macrospora*.

A similar disease of passion fruit called 'brown spot' has earlier been reported from Australia[1] and New Zealand[2] and caused by *Alternaria passiflorae* Simmonds. The disease caused by *A. macrospora*[3] on passion fruit reported in this communication is the first report from India. The culture has been deposited in the Commonwealth Mycological Institute, Kew, as IMI 196477.

The authors are grateful to Dr. G. S. Randhawa, Director, Indian Institute of Horticultural Research, Bangalore, for providing necessary facilities. Thanks are also due to Dr. Anthony Johnston, Director, and Dr. M. B. Ellis, of the Commonwealth, Mycological Institute, Kew, Surrey, England, for confirming the identity of the fungus.

Horticultural Experiment Station, BASANT RAM.
Chethalli 571 248, R. NAIDU.
Coorg, Karnataka, H. P. SINGH.
August 10, 1976.

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1. Smith, W. P. C., *J. Dep. Agric. W. Aust.*, 1939, 26, 445.
2. Brien, R. M., *N. Z. J. Sci. Tech.*, 1967, 21, 275.
3. Ellis, M. B., *Dematiaceous Hyphomycetes*, C.A.B., England, 1971, pp. 608.

IN VITRO FLOWERING OF SHORT APICES OF *BOUGAINVILLEA* × *BUTTIANA* CV. 'LOUISE WATHEN'

IN contrast to several reports of shoot apices growing *in vitro* as single vegetative shoots or proliferating into several shoots or callusing and giving rise to vegetative organs/plantlets[1], there are only a few instances of their flowering, *viz.*, *Cuscuta*[2,3], *Perilla*[4,5], *Pharbitis* and *Chrysanthemum*[6] and *Chenopodium*[7-9]. The shoot apex culture has been recommended[6,10], for better analysis of the biochemical nature of flower initiating/inhibiting substances, which is not yet fully understood. In the present communication are reported some preliminary results on *in vitro* flowering of shoot apices of *Bougainvillea* × *Buttiana* cv. 'Louise Wathen'.

Experimental

About 2 cm long shoot tips were taken from non-induced (vegetative) plants of *B.* × *Buttiana* cv. 'Louise Wathen', sterilized by treating with 0·1% HgCl$_2$ solution for 10–15 min. and thoroughly washed with sterile distilled water. From such surface-sterilized shoots, explants of about 2 mm long apices were excised and cultured in Murashige and Skoog's[11] nutrient agar medium supplemented with 0·8 mg/1 kinetin and 0·5 mg/1 IAA. The medium was adjusted to pH 5·8 and sterilized by autoclaving at 1·08 kg/cm^2 for 15 min. The cultures were incubated under 3000 lux fluorescent light for 15 hr. daily and at a regulated temperature of 27° ± 1° C.

The explants grew to rooted leafy shoots, which were maintained in aseptic culture by repeated subculture of their 2 cm long apices at 30-day intervals. After an incubation of 18 months, 20% of the cultures showed differentiation of bunches of flowers having good sized bracts of apricotorange orange colour similar to that found in nature (Fig. 1).

However, the shoots resulting from the cultures of nodal stem segments of the flowered shoots, produced flowers after only 2 months' incubation. The difference in time taken to flower by the two types of explants is noteworthy. Although it is not possible to give an exact explanation to this fact, it appears that some metabolic changes took place in the tissue of shoot during subcultures, and the initial period of 18 months might have been necessary for the accumulation of some flower inducing substance/substances to threshold level.

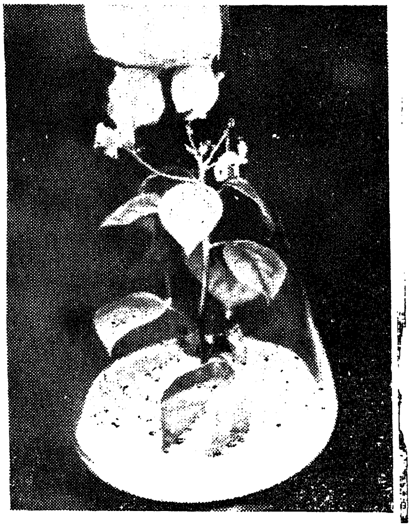

FIG. 1. An *in vitro* flowering shoot of *B.* × *Buttiana* cv. 'Louise Wathen'. × 0·6 approx.

The presence of such substance/substances in the subsequent explants taken from the *in vitro* flowered shoots facilitated flowering, hence the resulting shoots flowered in just 2 months. Such assumptions find support from the work reported by Chauard and Aghion[12] on tobacco, where explants of stem taken from a young plant or from the basal region of the flower bearing plant and cultured *in vitro* formed vegetative buds, but those excised from inflorescence stalk formed flower buds. Proximity of the explant to the inflorescence decided the ratio of flower buds to vegetative buds. Thus, they contemplated the existence of a gradient of some "floriginic factor" in a decreasing order from apex to base in a flowering tobacco plant.

We thank Dr. T. N. Khoshoo, Director, National Botanic Gardens, Lucknow, for the facilities.

Tissue Culture Laboratory, H. C. CHATURVEDI.
National Botanic Gardens, R. N. PRASAD.
Lucknow,
September 13, 1976.

1. Murashige, T., *Ann. Rev. Plant Physiol.*, 1974, 25, 135.
2. Loo, Shih-Wei, *Am. J. Bot.*, 1946, 33, 295.
3. Baldev, D., *Ann. Bot.*, 1962, 26, 173.
4. Raghavan, V. and Jacobs, W. P., *Am. J. Bot.*, 1961, 48, 751.
5. Chailakhan, M. KH., Butenko, R. G. and Lyubarskaya, I. I., *Fiziol. Rast.*, 1961, 8, 101.
6. Harada, H., *Nature*, 1967, 214, 1027.
7. de Fossard, R. A., *Bot. Gaz.*, 1972, 133, 341.
8. —, *Ibid.*, 1973, 134, 11.
9. —, *Ibid.*, 1973, 134, 103.
10. —, In: *Tissue Culture and Plant Science*, Ed., H. E. Street, Academic Press, Inc., N.Y., 1974, p. 193.
11. Murashige, T. and Skoog, F., *Physiol. Plantarum*, 1962, 15, 473.
12. Chauard, P. and Aghion, D., *C.r. Acad. Sci.*, 1961, 252, 3864.

SHORT SCIENTIFIC NOTES

On a Smooth Hammerhead Shark, *Sphyrna zygaena* (Linnaeus, 1758) New to Indian Waters

A mature female smooth hammerhead shark, *Sphyrna zygaena* (Linnaeus), 214 cm TL (Table I), was collected on 6-10-1975 from the hook and line fishing operated from the mechanised vessels off Porto Novo at a depth of 80-90 meters. Since hammerheads are identified from the shape of head and teeth structures only, the head has been preserved in the museum of the Marine Research Laboratory of Annamalai University, Porto Novo. No previous record of this species in Indian waters is available and so the present observation is of interest.

Range.—*S. zygaena* has been observed only in cool waters of northern and southern Hemispheres (Gilber Map 2) being reported to occur in the U.S., Europ Japan and Red Sea in the northern hemisphere an South America, South Africa, Australia and Ne Zealand in the southern Hemisphere. The prese observation extends its distributional record to Indi waters.

Remarks: No previous record of *Sphyrna zygae* has been made in Indian waters so far. Day[2] describ *Cestracion zygaena* from the Malabar coast which l later named as a junior synonym of *Zygaena malle* Fowler[4] and Misra[5] synonymised *Z. malleus* un

TABLE I

Morphometric characters of a female Sphyrna zygaena *expressed as per cent of TL*

Head width	26·3
Snout to 1st gill slit	16·8
pectoral	19·9
pelvic	46·5
Eye diameter	1·5
Between dorsal bases	28·3
nares	20·9
Mouth width	7·4
1st dorsal base	9·2
height	12·9
2nd dorsal base	3·1
height	1·9
Anal base	4·5
height	2·2
Pectoral base	6·2
length	16·8
Caudal upper lobe	26·8
lower lobe	14·2

S. zygaena but Day's *Z. malleus* clearly shows the median indentation on the anterior margin of head which is characteristically absent in *S. zygaena*.

The distribution of this species was reported to be continuous during pleistocene glacial periods when equatorial waters were cooler than at present bu when the glaciers withdrew and the tropical seas became warmer, this species was supposed to be confined to the cooler waters (*vide* Gilbert[1]). It is interesting that this species was landed in October during the north-east monsoon time when the sea water is generally cooler.

We thank Dr. A. J. Bass of National Museum, Wellington, New Zealand, and Dr. P. K. Talwar of the Zoological Survey of India, Calcutta, for their help and the University Grants Commission for financial support.

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1. Gilbert, C. R., "A revision of the hammerhead sharks (Family: Sphyrnidae)," *Proc. U.S. natn. Mus.*, 1967, **119** (3539). 31,
2. Day, F., *The Fishes of Malabar*, London, 1865, p. 270.
3. —, *The Fishes of India*, 1 and 2. William Dawsons and Ltd., 1878, p. 719, pl. 186, figure 4.
4. Fowler, H. W., "Contributions to the biology of the Philippine archipelago and adjacent region," *Bull. U.S. natn. Mus.*, 1941, **13** (100), 217.
5. Misra, K. S., "An aid to the identification of the common commercial fishes of India and Pakistan," *Rec. Indian Mus.*, 1959, **57**, 96.

Wet Rot Disease of Banana from Varanasi

A large number of Banana fruits were rendered unpalatable due to wet rot infection. The epicarp of the infected fruits turned sticky, wet and dark brown in colour whereas the endocarp, the edible portion, became reddish green on account of mycelial growth and fruiting structures.

The organism was isolated on PDA from the diseased fruits. The microscopic characters of the pathogen from a ten-day old culture confirmed that the pathogenicity was due to fungus. Based on morphological characters the present fungus was identified as *Botrytis cinerea* Pers.

The fungus is omnivorous and is capable of attacking numerous plants[1]. This is the first report of the pathogen causing wet rot disease in banana fruits.

One of us (GPS) is grateful to Dr. B. N. Srivastava, the then Principal of Govt. Degree College, Jakhini, Varanasi, for providing laboratory facilities and the constant encouragement.

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1. Subramanian, C. V., *Hyphomycetes*, 1971, p. 406.

A New Bulb Rot of *Allium cepa*

During the survey of local vegetable market, a hitherto unrecorded, severe, soft rot disease of Onion (*Allium cepa* L.) bulb was observed. The infected portion becomes macerated, soft with shrinkage and pale white in colour. The complete internal rotting was evident in 7–10 days.

The causal organism was isolated from the diseased bulb and identified as *Fusarium solani* (Mart.) Appel and Wr. The fungus grew well on potato dextrose agar and host pulp agar media. Healthy sterilised onion bulbs were inoculated with the fungus by an inoculum disc after pricking the bulb surface and also by spraying with inoculum. After 8 days the fruits showed disease symptoms. From the inoculated bulb the same fungus was re-isolated.

The authors are grateful to Prof. S. B. Saksena, Head, Department of Botany, University of Saugar, for providing facilities and also to C.S.I.R. for the award of Junior Research Fellowship to senior author.

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Phytopathogenic Fungi of Meerut : Some New Records for India

During the collections of phytopathogenic fungi from Meerut (India), the following new host records were established, the specimens of which have been deposited with Commonwealth Mycological Institute, Kew, England:

1. *Gercospora thunbergiana* Yen.

On living leaves of *Thunbergia grandiflora* (Acanthaceae), from Mall Road, Meerut, October 1975, IMI 199399. The present report is a new record for India.

2. *Uromyces setariae-italicae* Yosh.

On living leaves of *Panicum trypheron* (Graminae), from Meerut University Farms, January 1976, IMI 203304. Earlier it has been reported on a number of *Setaria* spp. Sydow and Butler[1]. However the present report is a new host record for India.

3. *Uromyces eragrostidis* Tracy.

On living leaves of *Eleusine indica* (Graminae), from Medical College Campus, Meerut, September 1975, IMI 203305. It has been reported earlier on the leaves of *Eragrostis cynosuroides* Sydow and Butler[2] but the present report is a new host record for India.

The authors are grateful to Drs. Mulder and Mordue of C.M.I., Kew, England, for identification.

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Meerut College, Meerut (India). P. N. SINGH.
September 30, 1976.

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1. Sydow, H. and Butler, E. J., *Ann. Mycol.*, 1906, **4**, 424.
2. — and —, *Ibid.*, 1907, **5**, 488.

Facultative Parthenogenesis in *Cyphoderus javanus* (Collembola: Insecta)

The information available on parthenogenesis in Collembola has been reviewed briefly by Marshall and Kevan[1], Christiansen[2] and Snider and Butcher[3]. About six species are known to reproduce obligatorily or facultatively by parthenogenesis. They belong to the families Hypogastruridae, Onychiuridae and Isotomidae. The present report is apparenlty the first clear instance of parthenogeneis in the family Entomobryidae.

Cyphoderus javanus was successfully cultured in the laboratory on yeast. Sexes in this species are identical in appearance and sexing based on external morphology is difficult. Indivuduals reared in pairs laid eggs. In some cases when one of the individuals died and the other happened to be a female, the latter reared alone, laid eggs uninhibited. One such female lived for about 100 days after the death of its mate and laid as many as 91 eggs. Another female lived for 25 days after the death of its mate and laid 42 eggs during this period. This observation suggested the existence of parthenogenesis in *C. javanus*. Five eggs laid by a single female were, therefore, reared in isolation in new culture chambers. The individuals hatching out from these eggs reached maturity and two of them laid eggs. One female laid 39 eggs in five batches and the second one laid 6 eggs in one batch. The clutch size was 6–7 eggs. The eggs were transferred to separate culture chambers. The individuals emerging from the eggs were kept for about a month but none of them laid eggs. Presumably all of them were males. It may be possible that in the first set of experiments, the eggs laid were fertilized as spermatophores laid by the male before its death were available in the substratum. This possibility demanded further evidence for parthenogenesis in *Cyphoderus* which is provided by the isolation experiments. It appears that *C. javanus* is capable of facultative parthenogenesis producing males. Apparently when parthenogenetic eggs are laid the clutch size is small being 6–7 compared to the condition when the eggs are laid after fertilization (*ca* 14 eggs in a clutch).

We are thankful to Professor K. M. Alexander for facilities in the Department.

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1. Marshall, V. G. and Kevan, D. K. McE., *Canda. Ent.*, 1962, **94**, 575.
2. Christiansen, K., *Ann. Rev. Entomol.*, 1964, **9**, 147.
3. Snider, R. M. and Butcher, J. W., *Great Lakes Entomol.*, 1973, **6** (4), 97.

Abnormal Mitosis, Scattered Metaphase and Polyploidy Induced by Chlorflurenol in *Vicia faba*

2-Chloro-9-carboxylic acid, commonly known as Chlorflurenol (ISO) is the most active morphactin tested so far. Despite their high morphogenetic potency[1] morphactins have been left untouched from cytological standpoint. The present study concerns with effect of chlorflurenol on the mitotic cell division.

TABLE I

Frequency of cells in different stages (in per cent) of the total dividing cells

Duration of treatment	Mitotic index	Prophase	Scattered metaphase	Metaphase plate	Anaphase	Telophase/ binucleate	Tetraploid	Unequal separation/ laggards	Multipolar mitosis	Somatic reduction	Chromosome clumping
1 hour	6·4	39	13·8	11·7	4·7	9·3	17	1·0	3	0·5	..
2 hours	4	56	13·4	2·7	1·4	7	11·9	1·8	2·2	1·4	2·2
3 hours	1·5	47	18	2	2	4	4	1·0	..	..	22

Germinating seeds of *Vicia faba* with the roots of about one cm in length were immersed in aqueous solution (0·05%) of chlorflurenol 7301 (Celamerck, 6507 Ingelheim, Germany) at 25 ± 1° C for 1, 2 and 3 hours durations separately. Treated seeds were thoroughly washed and allowed to grow half dipped in water. After three hours' recovery period in each case, the roots were fixed in Carnoy's solution. Root tips were feulgen squashed for cytological observations. In all cases, the count determinations were based on approximately 500 dividing cells.

Chlorflurenol inhibits mitosis and arrests the dividing cells at metaphase causing scattering of metaphase chromosomes. However, it allows a certain percentage of cells to proceed further like colchicine[2] resulting in tetraploidy and binucleate cells followed by failure of cytokinesis. Of the various abnormalities induced by the treatment involves unequal anaphase separation, lagging chromosomes, multipolar mitosis, somatic reduction, chromosome clumping, etc. The cells showing anaphase separation of chromosomes without kinetochore separation and bipartitioning into chromatids (*i.e.*, somatic reduction[3]), suggest that chlorflurenol interferes with glycolytic enzymes and thus inhibits the cleavage of kinetochore, similar to the glycolytic inhibitors (*e.g.*, iodoacetic acid, iodoacetamide)[4]. Preliminary studies using other plants *e.g.*, *Allium cepa*, *Hordeum vulgare* show that chlorflurenol may be used as a pretreating agent in chromosome work. Results are documented in Table I.

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1. Sankhla, N., Bohra, S. P., Vyas, S. P. and Sankhla. D., In H. Y. Mohan Ram, J. J. Shah and C. K, Shah, (eds.), *Form, Structure and Function in Plants*, Sarita Prakashan, Meerut, 1975, p. 255.
2. Sharma, A. K. and Ghosh, S., *Acta Biol. Acad. Sci. Hung.*, 1969, 20, 11.
3. — and Sharma, A., *Chromosome Techniques: Theory and Practice*, Butterworth, London, 1972.
4. Wilson, G. B. and Morrison, J. H., *The Nucleus*, 1958, 1, 45.

REVIEWS AND NOTICES OF BOOKS

Annual Review of Astronomy and Astrophysics, Vol, 14. (Edited by Geoffrey R. Burbidge, David Layzer and John G. Phillps. (Annual Reviews, Inc., 4139 El Camino Way, Palo Alto, California 94306, USA), 1976. Pp. 500. Price: U.S.A. $ 17.00, Elsewhere: $ 17.50).

In these days of rapid growth in various branches of theoretical and observational astronomy, there is every need for comprehensive review articles to keep the knowledge of the practising astronomer up-to-date. The series of *Annual Review of Astronomy and Astrophysics* are filling such a need admirably since their inception and the present volume is no exception.

The fourteenth volume contains 17 articles covering a wide spectrum. Two articles deal with instrumentation – Echelle spectrosocpy with emphasis on its astronomical applications and the young and rapidly growing field of Radio Astrometry, which has applications in Geodesy besides dynamical astronomy. Three articles are concerned with solar system studies —Chemical composition of solar primitive material, physical processes in comets, and orbital resonances of satellites. Four more are devoted to stars — stellar opacity, the structure of cataclysmic variables and nonradial oscillations.

The interstellar medium is dealt with in two articles, one on magnetic fields, and the other on hydrogen line studies. There are two articles on other aspects of the galaxy — one on its chemical evolution and the other on galactic soft x-ray sources.

The last five articles cover the fields of extragalactic sources and cosmology. They deal with line emission from quasi-stellar sources, radio emission from spiral galaxies and extended extragalactic radio sources, radio and optical properties of BL Lacertae objects and observational tests of antimatter cosmologies.

Like most of the preceding volumes in this series, the present one is a must for any library catering to astronomers or astrophysicists.

CH. V. SASTRY.

A Text Book of Geology. By S. K. Chadda. (Kapoor Brothers, Jammu–Srinagar, Kashmir), 1976. Pp. viii + 300. Price: Rs. 15·00.

The book is a welcome addition to a few books on Geology written recently by Indian authors. The book has 28 chapters covering probably the syllabus of degree colleges in Jammu-Srinagar area. The titles of some chapters do not sound well (Ex. :- Water working under the ground). There is a chapter on soils in general and on the soils of India, though brief in coverage. Some chapters have the heading, "Study of......". There is no consistency in giving proper titles to the various chapters.s. Chapter dealing with 'Stratigraphy' covers 'geological record of India' which is not complete as it ends with the Upper Gondwana System. This is one of the serious drawbacks of the book. An index at the end has contributed something to the value of the book. At the end of each chapter is given a set of 'typical questions' to probably justify the book being a 'text book'. The material or quantum of information given to serve the interests of those studying Civil Engineering and Technology is not sufficient. The author's attempt to give some illustrations from the Indian region is laudable, though mainly all of them are poor in quality and many more illustrations could have been selected from the Indian region to replace those from other countries. The book has a clear imprint of "notes book" with plenty of awkwar expressions. A language expert would have been great use to improve the language of this book. Th price of the book is quite reasonable. The boo would become more useful if improvemen are attempted in the next edition.

S. SAMBE GOWD

Selected Topics in Environmental Biology. Editors: Bhatia, G. S. Chinna and Baldev Singh. (Published Interprint Publications, New Delhi), 1975. Pp. + xiv. Price: Rs. 300.

In the recent years, there has been intensification activities in many countries on the studies relating interactions between man and his environment. Th is due not only to the urge to prevent human sufferin and disease and to maximise human output, but al to expand the limits of habitable regions necessitat by the pressure of rapidly increasing population.

Environmental biology has been rightly conside to be of sufficient interest for a discussion by the Int national Congress of Physiological Sciences.

The present volume is based on the Symposia and papers presented at the XXVI International Congr of Physiological Sciences held at New Delhi, Octob 20–26, 1974. The papers presented in the volu demonstrate world wide interest and effort in resear related to environmental biology and the wide g in our knowledge of the subject.

Symposia were arranged on the subjects of h altitude physiology, underwater physiology, phys logical effects of cold, physiological effects of heat a physical and physiological effects of accelerations. T other topics discussed have been classified and prese under the following sections: Thermoregulation, C stress, Heat stress, Hypothermia and Thermal inju Adoptation to stress and physical work, Prolon exposure and short term exposure to hypoxia, Pul nary oedema, Constitution and body function different ethnic groups, Speed acceleration and gra Environmental pollution, Noise, Hyperbaric Emotional stress and Cyclic variations in body f tions.

This volume is certainly a valuable reference for those concerned with the quality of environ and the effect of stress on human health.

M.

6270/76. Published by S. R. S. Sastry, Manager, Current Science Association, Bangalore-560006
and Printed at The Bangalore Press, Bangalore-560018.

A TEST FOR THE USE OF TWO-FOLD TORSIONAL POTENTIAL FOR Ψ ROTATION—'UNPERTURBED DIMENSIONS OF POLYPEPTIDES'

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RECENTLY, by applying classical energy methods to polypeptides, a number of interesting results have been reported[1–9]. The calculated values[9] of C_α are found to be sensitive to the input peptide geometry. These studies also showed no correlation between C_α and the number of allowed conformations of the peptide unit suggesting that the steric factor σ need not always provide information about the flexibility or freedom of rotation of chain units. In the classical potential energy approximation the energy of a system is divided into several discrete contributions, such as Vander Waals energies, electrostatic interactions, torsional energy, hydrogen bond energy, etc. Though the form and nature of the functions used by different groups[1–5] for computing non-bonded energy differ, to some extent but all of them predict same trends in the energy contours including minima at about the same region. On the other hand, the form of potential functions that have been used by different workers for computing torsional energy differ drastically. For the potential function associated with ϕ (C′—N—C$^\alpha$—C′) both the forms $V_0(\phi)/2\ (1 + \cos 3\phi)$ and $V_0(\phi)/2\ (1 - \cos 3\phi)$ have been used. Values of 0·6 to 1·5 kcal.mole^{-1} have been used for $V_0(\phi)$. Similarly for the potential function associated with ψ (N—C$^\alpha$—C′—N) the earlier investigators have used a form $V_0(\psi)/2\ (1 + \cos 3\psi)$, with low values ranging from 0·25 to 1 kcal.mole^{-1} for $V_0(\psi)$. Recently from quantum chemical studies on model compounds Sasisekharan and co-workers[10] have suggested that the potential function associated with ψ should be of the form $V(\psi) = V_0(\psi)/2\ (1 - \cos 2\psi)$ but not $V_0(\psi)/2\ (1 + \cos 3\psi)$. These authors suggested a value of 4 kcal.mole^{-1} for $V_0(\psi)$ from theoretical studies, without any experimental confirmation. Infrared studies of amides made by Shimanouchi[11] also support the two fold torsional function for $V(\psi)$. For the potential function associated with ϕ (C′—N—C$^\alpha$—C′), these authors also suggested that there is no need for separately adding the $V(\phi)$ term in empirical potential energy calculations since $V(\phi)$ was found to be very small[12]. The implications of these changes in the form of intrinsic torsional functions have been discussed by Ramachandran[13], while dealing with the ordered polypeptide chains. In this paper, the authors have examined the relevance of the new form of intrinsic torsional potential functions on the unperturbed dimensions of polypeptides.

Fixing of Atoms and Calculation of Potential Energy

The averaged peptide parameters reported by Ramachandran *et al.*[14] have been used for calculations on poly (L-alanine). The side groups were fixed by the method suggested by Ramachandran and Sasisekharan[1] assuming C$^\alpha$—H$^\alpha$ and C$^\alpha$—C$^\beta$ bond lengths to be 1·0 and 1·54 Å respectively. The —CH$_3$ was treated as an united atom with higher Vander Waals radii.

The conformational energy was computed using the expression,

$$V(\phi_i, \psi_i) = V_0(\phi)/2\,(1 + \cos 3\phi_i) + V_0(\psi)/2 \times (1 - \cos 2\psi_i) + \sum_{k,l} V_{k,l}(\phi_i, \psi_i) + \sum_{k,l} V_c\, k, l \tag{1}$$

Values of 0–1·5 kcal.mole^{-1} for $V_0(\phi)$ and 1–4 kcal.mole^{-1} for $V_0(\psi)$ (in independent calculations) have been used. 6-exp non-bonded potential functions[1] with a set of recently modified constants[15] for H···H and H···X interactions were used to compute the non-bonded interaction energy $[\sum V_{k,l}(\phi_i, \psi_i)]$. These calculations also were repeated with the constants suggested by Flory and co-workers[6] for non-bonded potentials. Electrostatic energy $(\sum_{k,l} V_{c\,k,l})$ was computed using the expression reported by Brant *et al.*[7] and the charges reported by Srinivasan and Rao[9]. The characteristic ratios (C_α) of an infinitely long chain were obtained by using the expression,

$$C_\alpha = [(E + \langle T \rangle)(E - \langle T \rangle)^{-1}]_{22} \tag{2}$$

where E is the identity matrix of order 3 and $\langle T \rangle$ is the statistical-mechanical averaged matrix. The subscript 22 denotes the (2, 2) element of the final matrix. The averaged matrix $\langle T \rangle$ was evaluated as described by Srinivasan and Rao[9].

RESULTS AND DISCUSSION

Since Ramachandran *et al.*[14] suggested a value of about 110° for the bond angle (τ) at non-glycyl α-carbon atom from crystal structure data, most of the calculations were carried out for this (τ) angle and these results are presented in Table I. It is seen from the table that the magnitude of $V_0(\psi)$ and $V_0(\phi)$ do affect the values C_α significantly. The value C_α

TABLE I

Computed C_α values for Poly (L-alanine) using a vo-fold function for $V(\psi)$ with different $V_0(\psi)$ values ($\tau = 110°$ and $T = 298°$ K has been used)

Nature of functions used	C_α values $V_0(\psi)$ = (in kcal. .mole^{-1}) 1	2	3	4
(N, Z)	9·0	9·3	9·9	10·3
(N, TA)	7·9	8·2	8·6	9·0
(N, TB)	7·0	7·2	7·5	7·9
(N, TC)	6·4	6·3	6·6	7·0
(F, Z)	16·9	16·8	19·6	23·6
(F, TC)	12·0	11·6	12·3	14·0

N—Non-bonded potential functions and constants given in Refs. 1 and 15.

F—Non-bonded potential functions and constants given in Ref. 7.

Z—$V_0(\phi) = 0$

TA—$V(\phi) = 0·25\,(1 + \cos 3\phi)$

TB—$0·5\,(1 + \cos 3\phi)$

TC—$0·75\,(1 + \cos 3\phi)$.

(10·3) obtained assuming $V_0(\phi) = 0$ and $V_0(\psi) = 4$ kcal. mole^{-1} (as suggested by Kolaskar *et al.*[10,12]) is slightly higher than the experimental value of 8·6 ± 0·5[16–18]. On the other hand the value 8·6 obtained with $V_0(\phi) = 0·5$ kcal. mole^{-1} and $V_0(\psi) = 3$ kcal. mole^{-1} in the expression agree well with the experimental data. This suggests that the corrected barrier $V_0(\psi)$ is slightly lower than the 4 kcal. mole^{-1} suggested by Kolaskar *et al*[10]. Similarly the value of $V_0(\phi) = 0·5$ kcal. mole^{-1} suggests that the intrinsic barrier about N—C^α is small in agreement with the recent theoretical results of Kolaskar *et al.*[12]. Though these authors suggested a complete neglect of the $V(\phi)$ term in the empirical potential energy calculations, the present calculations suggest the retention of this term with a low intrinsic barrier. The values of C_α, obtained with the constants suggested by Brant *et al.*[7] for the non-bonded potentials, are very high compared to the experimental value[16–18] of 8·6. The large differences in the computed C_α values arise mainly due to differences in non-bonded potentials involving H-atom. Unlike Brant *et al.*[7] Ramachandran[12] suggested softer potentials for H....H and H....X interactions from the experimental observations in the solid state. In fact, the observed conformational parameters for the LL-bend were explained[12] better with modified non-bonded potentials. The present study also supports the use of new form of potential function for $V(\psi)$, *i.e.*, $V(\psi) = 1·5\,(1 - \cos 2\psi)$ together with the softer potentials for H ··· H and H··· X interactions for conformational energy studies on polypeptides.

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1. Ramachandran, G. N. and Sasisekharan, V., *Advan. Protein Chem.*, 1968, **23**, 283.
2. Wiliams, D. E., *J. Chem. Phys.*, 1966, **45**, 3770.
3. Scheraga, H. A., *Advan. Phys. Org. Chem.* 1968, **6**, 103.
4. Momany, F. A., Vanderkooi, G. and Scheraga, H. A., *Proc. Nat. Acad. Sci. U.S.*, 1968, **61**, 429.
5. Kitaigorodsky, A. I., In: *Advances in Structure Research by Diffraction Methods*, Vol. 3, R. Brill and R. Mason, eds., Pergamon Press, Oxford, 1970. p. 173.
6. Brant, D. A. and Flory, P. J., *J. Am. Chem. Soc.*, 1965, **87**, 2791.
7. —, Miller, W. G. and Flory, P. J., *J. Mol. Biol.*, 1967, **23**, 47.
8. Srinivasan, A. R. and Rao, V. S. R., *J. Polym. Sci.*, 1972, A-2, **10**, 693.
9. — and —, *Pramāna*, 1975, **4**, 95.
10. Kolaksar, A. S., Sarathy, K. P. and Sasisekharan, V., *Curr. Sci.*, 1975, **44**, 35.
11. Shimanouchi, T., *Discuss. Far. Soc.*, 1970, **49**, 60.
12. Kolaskar, A. S., Sasisekharan, V. and Sarathy, K. P., *Theoret. Chim. Acta (Ber.)*, 1975, **38**, 109.
13. Ramachandran, G. N., *J. Indian Inst. Sci.*, 1974, **56**, 375.
14. —, Kolaskar, A. S., Ramakrishnan, C. and Sasisekharan, V., *Biochim. Biophys. Acta*, 1974, **359**, 298.
15. —, personal communication.
16. Brant, D. A. and Flory, P. J., *J. Am. Chem. Soc.* 1965, **87**, 2788.
17. Furita, H., Teramoto, A., Yamashita, T., Okita, K. and Ikeda, S., *Biopolymers*, 1966, **4**, 781.
18. Terbujevich, M., Peggion, E., Cosani, A., D'Este, G. and Scoffone, E., *Eur. Polym. J.*, 1967, **3**, 681.

MÖSSBAUER STUDY OF THE IRON (III) COMPLEXES OF PYROPHOSPHORIC ACID

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ABSTRACT

The complexes of iron (III) with pyrophosphate both in acidic and alkaline medium were isolated. Their chemical formulae were established using chemical analysis data. The Mössbauer spectra of the two complexes, *i.e.*, $H[Fe(H_2P_2O_7)_2(H_2O)_2].4H_2O$ and $Na_5[Fe(P_2O_7)_2(H_2O)].3H_2O$ gave the same values of Isomer shift. This fact go to show that the electron density in both the complexes is roughly the same but the difference in quadrupole splitting of the two type of complexes predicts that the symmetry around the ferric ion in case of complex formed in acidic medium is more distorted than in the alkaline medium.

INTRODUCTION

LITERATURE survey on metal pyrophosphates[1-8] would reveal that almost all the investigations have been carried out with the products obtained by the reaction of sodium pyrophosphate with the metal salts. In view of the fact that sodium pyrophosphate solution cannot exist in a stable form[9-10], the results achieved by such studies are likely to be less reliable. However, if studies on metal pyrophosphates are carried out by using pyrophosphoric acid, more meaningful data can be achieved. Also, no Mössbauer spectral studies either in the case of pyrophosphoric acid or sodium pyrophosphate complexes have been reported so far. In the present communication the Mössbauer study of the complexes of iron (III) with pyrophosphoric acid as well as with sodium pyrophosphate is presented.

EXPERIMENTAL

Reagents and Solutions.—All the reagents employed were of A. R. quality. The pyrophosphoric acid and sodium pyrophosphate used were of MERCK Products. All the solutions were made in double distilled water.

Preparation of the Complexes.—Solutions of ferric nitrate and pyrophosphoric acid (Conc. 1·0 M each) were mixed in the molar ratio of 1 : 2 and allowed to stand for about 2 hours. The precipitate obtained was filtered, washed several times with water and then dried at 90° C. The complex of iron (III) with $Na_4P_2O_7$ was prepared similarly.

The spectra of the two type of complexes were run by constant Acceleration Velocity Drive Mössbauer Spectrometer.

RESULTS AND DISCUSSION

From the chemical analysis, the molecular formulae of the two complexes were established to be $H[Fe(H_2P_2O_7)_2(H_2O)_2].4H_2O$. and $Na_5[Fe(P_2O_7)_2(H_2O)_2].3H_2O$.

Mössbauer Study of $H[Fe(H_2P_2O_7)_2(H_2O)_2].4H_2O$. The observed values of Isomer shift and Quadrupole splitting (Fig. 1) are 0·433 mm/sec. (w.r. to Fe) and 0·671 mm/sec. respectively. Iron is bonded through Fe–O bonding with its ligands and the symmetry formed by the surrounding ligands is octahedral with a covalent bonding and iron exists in Fe (III) form. The values of I.S. and Q.S. observed supports the view that iron is in Fe (III) form and the symmetry around iron is octahedral with a little distortion due to different ligand groups (P–O–H, O–P–O) responsible to octahedral symmetry. The infra-red studies go to show that the stretching frequencies of the P–O–H and O–P–O groups are shifted on complexation and thereby these groups are responsible for distortion. Further magnetic moment values which comes out to be 5·83 B.M., confirms that it is a high spin Fe (III) complex.

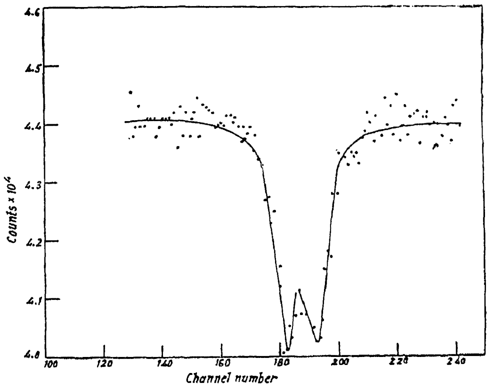

FIG. 1. Mössbauer spectrum of $H_2[Fe(H_2P_2O_7)_2(H_2O)_2]\cdot 4H_2O$.

Study of $Na_5[Fe(P_2O_7)_2(H_2O)_2].3H_2O$.—The observed values of I.S. and Q.S. are, $\delta = 0{\cdot}44$ mm/sec. (w.r. to Fe) and $\Delta = 0{\cdot}46$ mm/sec.

As discussed in the study of $H[Fe(H_2P_2O_7)_2(H_2O)_2].4H_2O$, here too, iron is in Fe (III) state with octahedral surrounding and the ligand groups are bonded through covalent bonding forming $Na_5[Fe(P_2O_7)_2 (H_2O)_2].3H_2O$.

Comparison of the Spectra.—Mössbauer spectra give the same values of Isomer shift in the two cases, *i.e.*, 0·433 mm/sec. in the case of pyrophosphoric acid complex and 0·44 mm/sec. in the sodium pyrophosphate complex. This goes to show that the electron density in both the phosphate complexes is roughly the same but the difference in quadrupole splitting in these two types of complexes shows that the symmetry around ferric ion in pyrophosphoric acid complex is more distorted than in the complex of sodium pyrophosphate.

1. Rogers, L. B. and Reynolds, C. A., *J. Am. Chem. Soc.*, 1949, 71, 2081.
2. Subrahmanya, R. S., *J. Indian Inst. Sci.*, 1956, 38 A, 87.
3. Banerjee, S and Mitra, S. K., *Sci. and Cult.*, 1951, 16, 530.
4. Friksson, E. and Hogskol, K. L., *Ann.*, 1949, 16, 39.
5. Watters, J. I. and Kolthoff, I. M., *J. Am. Chem. Soc.*, 1948, 70 (23), 2455.
6. Vaid, J. and Ramachar, T. L., *Curr. Sci.* 1954, 23, 396.
7. Haldar, B. C., *Nature*, 1950, 166, 744.
8. Mustaev, A. K. and Yakdovenko, T. L. *Issled. Khim. Redkikh Soputstuuyushchikh Elem. Akad. Nauk. King. SSR, Inst. Neurg. Fig. Khim.*, 1966, 66, 69339 X, (Russ.), 82.
9. Growther. J. P. and Westman, A. E. R., *Cand. J. Chem.*, 1954, 32, 42.
10. Vanwazer, J. R., Griffith, E. J. and McCullough, J. F., *J. Am. Chem. Soc.*, 1955, 77, 287.

CENTRAL STIMULANT ACTIVITY OF A BENZOCYCLOHEPTENE DERIVATIVE IN MICE: COMPARISON WITH (+) AMPHETAMINE

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ABSTRACT

7-Dimethylamino-5, 6, 8, 9-tetrahydro-7-phenyl-7H-benzocycloheptene hydrochloride has been tested for its central stimulant activity by using two models in mice. This compound increases spontaneous locomotor activity and antagonises reserpine-induced catalepsy and hypothermia. The results reveal that it could possibly stimulate the central nervous system like (+) amphetamine.

INTRODUCTION

PRELIMINARY studies with the compound, 7-Dimethylamino-5, 6, 8, 9-tetrahydro-7-phenyl-7H-benzocycloheptene hydrochloride[**] showed that it could stimulate the central nervous system in mice and hence it was decided to test this property at three dose levels in two models, (1) on spontaneous locomotor activity, (2) against reserpine-induced catalepsy and hypothermia. Results obtained in such a study provide the basis for this report.

MATERIALS AND METHODS

(a) *Stimulation of Spontaneous Locomotor Activity in mice*

Spontaneous locomotor activity was taken as a parameter of behavioural excitation[1]. It was recorded by means of a conventional light beam cage[2,3]. The light and sound-proof cabinet contained 4 identical cages, each 22 cm × 37 cm × 8 cm, crossed by 6 light beams. Five mice (18–25 g) were placed in each cage 15 min. after subcutaneous administration of the compound/vehicle and the number of light beam interruptions taking place was recorded at 30 min. intervals for 7 hours, through an automatic counter. Experiments were repeated 6 times, giving a total of 30 mice per treatment. The mean total 'motility count for 5 mice over the recording period, and the mean, and Standard error of mean were calculated. Statistical comparisons were carried out by means of Student's '*t*' test.

The experiments were performed on male albino mice (NMRI Strain). The animals were kept under standard laboratory conditions (constant temperature 22 ± 1° C, humidity 60% and light on between 8 a.m. and 8 p.m.) and had no prior experience in the activity cages.

The substance was dissolved in one or two drops of glacial acetic acid and the pH was adjusted to 5–6. Control animals received the vehicle at the same pH. Three doses of the substance, 2·5, 5 and 10 mg Kg^{-1} were studied.

For comparison purposes, (+) amphetamine sulphate was administered subcutaneously at similar doses and its stimulant activity on locomotion was studied in a similar manner. (+) Amphetamine sulphate was dissolveed in 0·9% sodium chloride solution and used.

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A total of 240 mice were used for this study, 120 for each compound.

(b) Antagonism of Reserpine-induced Catalepsy in Mice

Mice (18–25 g) in groups of 10 were injected intraperitoneally with reserpine (5 mg Kg^{-1}) 17 h before intraperitoneal administration of the compound under investigation. Catalepsy was assessed at 30 min, 1 h, 2 h, 3 h, and 5 h by an observer who did not know the treatment using the method of Zetler and Moog[4]. The antagonism of catalepsy, *i.e.*. the ability to walk off a twine-covered vertical pole in a co-ordinated manner, was recorded. Control animals retain their position when placed on the side of the pole. The rectal temperature was recorded by a heat-sensitive thermistor probe. The probe was inserted to a depth of 3 cm and retained *in situ* until a constant temperature was displayed on the telethermometer.

The effects of three doses of the compound, 0·1, 1 and 10 mg Kg^{-1} were studied. 4 groups of 10 mice each were used for this study. One group served as a control.

RESULTS

(a) Stimulation of Spontaneous Locomotor Activity in Mice

The benzocycloheptene derivative caused a dose-dependent and significant stimulation of spontaneous activity (Table I). Maximum stimulation was observed 1 to 2·5 h after its administration. The increase in activity was stastistically significant till 5·5 h in the case of 10 mg Kg^{-1}, 3 h with 5 mg Kg^{-1} and 2 h with 2, 5 mg Kg^{-1}.

TABLE I

The effect of the benzocycloheptene derivative on the spontaneous locomotor activity in mice. Results are expressed as the mean number of light beam interruptions of a group of 5 mice for 7 h period, with Standard error of the mean. Six groups of 5 mice were investigated for each treatment and dose level. Experiments commenced 15 min after drug administration

Treatment in mg Kg^{-1} SC	No. of counts for 7 h ± S.E.M.	Statistical significance compared to control
Control	8017 ± 2121	
Benzocycloheptene derivative 2·5	15647 ± 4933	$P < 0{\cdot}01$
,, 5·0	24217 ± 3687	$P < 0{\cdot}001$
,, 10·0	31797 ± 5474	$P < 0{\cdot}001$

(+) Amphetamine sulphate stimulated locomotion from 2·5 mg Kg^{-1} in a dose-dependent (Table II). Increase in locomotor activity lasted nearly 3 hours. The activity was significant till 3·5h in the case of 10 mg Kg^{-1}, till 3h with 5 mg Kg^{-1} and till 2h with 2·5 mg Kg^{-1}.

TABLE II

The effect of (+) amphetamine sulphate on the spontaneous locomotor activity in mice. Other details are as given in Table I

Treatment in mg Kg^{-1} SC	No. of counts for 7 h ± S.E.M.	Statistical significance compared to control
Control	7010 ± 2475	
(+) Amphetamine sulphate 2·5	16488 ± 4758	$P < 0{\cdot}01$
,, 5·0	22394 ±7677	$P < 0{\cdot}001$
,, 10·0	23826 ±9198	$P < 0{\cdot}01$

The percentage increase in spontaneous locomotor activity between (+) amphetamine sulphate and benzacyloheptene derivative has been compared in Table III.

TABLE III

Percentage increase in spontaneous locomotor activity: comparison between (+) amphetamine and benzocycloheptene derivative

Doses mg Kg^{-1}	Percentage increase when compared to control	
	(+) amphetamine sulphate	Benzocycloheptene derivative
2·5	135	95
5·0	219	202
10·0	240	297

(b) Antagonism of Reserpine-induced Catalepsy in Mice

The compound exhibited a dose dependent antagonism of reserpine-induced catalepsy and hypothermia. The antagonistic activity started 30 min after the drug administration and the peak effect was observed at 60 min and started declining afterwards. The antagonism existed till 1 h at a dose of 0, 1 mg Kg^{-1}. At doses, 1 and 10 mg Kg^{-1}, the antagonism was observed till 2 h and 5 h respectively.

DISCUSSION

The benzocycloheptene derivative and (+) amphetamine sulphate have been shown to produce dose-dependent stimulation of spontaneous locomotor activity in mice. The intensity of stimulation was almost same between (+) amphetamine sulphate and the compound under investigation over a period of 7 h. The stimulation of locomotor activity by (+) amphetamine sulphate has been attributed to be mediated by brain dopamine[5-7] and noradrenaline[8-10].

Reserpine-induced cataleptic state is said to be due to the depletion of catecholamines and serotonin in the central nervous system. (+) Amphetamine sulphate antagonises the cataleptic state of reserpine by stimulating the adrenergic[11] and serotoninergic receptors[12]. Van Rossum *et al.*[13] has presented evidence for a direct action of (+) amphetamine on adrenergic receptors in brain.

Because of the above considerations, it may be possible that the central stimulant activity of the new compound, a derivative of benzocycloheptene, could involve the mediation of the transmitters, catecholamines and serotonin as in the case of (+) amphetamine sulphate or by a different mechanism. Further experiments are necessary to establish the suggested mechanisms.

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1. Smith, C. B., *J. Pharmacol. Exp.Ther.*, 1965, 147, 96.
2. Johnson, A. M., Loew, D. M. and Vigouret, J. M., *Br. J. Pharmac.*, 1976, 56, 59.
3. Stula, A. D. and Meier, M., *Neuropharmac.*, 1976, 15, 383.
4. Zetler, G. and Moog, E., *Naunyn Schmeidebergs. Pharmak.*, 1958, 232, 442.
5. Costa, E., Gropetti, A. and Naimzada, M. K., *Br. J. Pharmac.*, 1972, 44, 742.
6. Van Rossum, J. M. and Hurkmans, J. A. T. M., *Int. J. Neuropharmac.*, 1964, 3, 227.
7. —, *Int. J. Neurobiol.*, 1970, 12, 307.
8. Svensson, T. H. and Waldeck, B., *Psychopharmacologia, Berl.*, 1970, 18, 357.
9. Maj, T., Grabowska, M. and Gajda, C., *Eur. J. Pharmac.*, 1972, 17, 208.
10. Anden, N. E., Strömbom, U. and Svensson, T. H., *Psychopharmacologia, Berl.*, 1973, 29, 289.
11. Innes, I. R. and Nickerson, M., *The Pharmacological Basis of Therapeutics*, Eds. Goodman, L. S. and Gilman, A., The Macmillan Company, London, 1971, p. 503.
12. Vane, J. R., *Adrenergic Mechanisms* (a Ciba foundation symposium), Eds. Vane, J. R., Wolstenholme, G. E. W. and O'Conor, Maeve., J. and A. Churchill Ltd., London, 1960, p. 356.
13. Van Rossum, J. M., Van der Schoot, J. B. and Hurkmans, J. A. T. M., *Experientia*, 1962, 18, 229.

SOME ADDITIONS TO THE LICHEN FLORA OF INDIA

I. Genus *Hypotrachyna* (Vain.) Hale (Parmeliaceae)

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IN our on-going research project on the lichen flora of Western Ghats (south western India) we have so far determined sixty-nine species belonging to the genera *Bulbothrix, Hypogymnia, Hypotrachyna, Menegazzia, Parmelina, Parmotrema, Parmelia, Pseudoparmelia, Relicina* and *Xanthoparmelia* of the family Parmeliaceae. Careful search of the literature revealed that of these sixty-nine species twenty-two taxa were not previously recorded under the composite genus *Parmelia* Ach. (Awasthi, 1965[1]) from Indian subcontinent. Six species of the genus Hypotrachyna which constitute additions to the lichen flora of India are detailed in this note. Chemical studies were carried out by thin-layer chromatography (TLC) using EMerck precoated silica gel F_{254} aluminium plates and B.D.A. and H.E.F. solvent systems (Culberson, 1972[2]). Specimens referred to in the text are deposited in the Lichen unit of the Ajrekar Mycological Herbarium (AMH).

The genus *Hypotrachyna* (Vain.) Hale is one of the recently segregated genera from the old composite genus Parmelia Ach. (Hale, 1974[3]). It is characterised by non-ciliate thallus and dichotomously branched rhizines.

1. *Hypotrachyna costaricensis* (Nyl.) Hale = *Parmelia costaricensis* Nyl.

Thallus corticolous, closely adnate to the substratum, 2–4 cm broad; lobes sublinear to linear, 2–3 mm broad, isidiate, isidia cylindrical, erect, simple to branched; lower surface black to the margin, rhizinate; rhizines dense, dichotomously branched; apothecia not seen.

Chemistry : Cortex K + yellow, medulla K –, C –, KC –, P –. Atranorine and Fatty acids present.

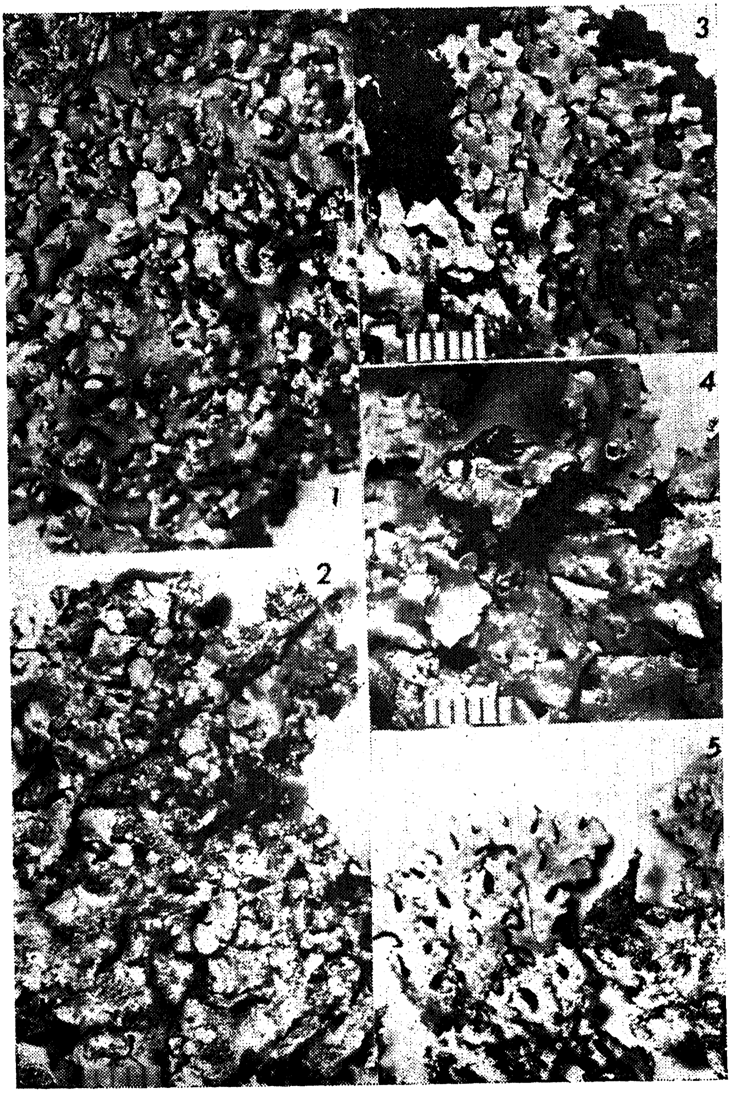
Specimens examined : 75·434 Silver Cascade, Kodaikanal, Tamil Nadu; 76·789 Munnar—Devicolam Road, Kerala.

Geographical Distribution : West Indies, Central America, South America, Philippines, Indonesia, Malaysia.

2. *Hypotrachyna crenata* (Kurokawa) Hale
= *Parmelia crenata* Kurokawa

Thallus saxicolous, 4–5 cm broad; lobes subirregular, 3–4 mm broad, margins crenate, lobes moderately isidiate, isidia short, mostly branched; lower surface black in the centre with faint brown peripheral zone, rhizines sparse; apothecia not seen.

Chemistry : Cortex K + yellow, medulla K + yellow, C –, KC –, P + pale orange. Atranorine, stictic, constictic acid and 2 unknown substances present.



FIGS. 1–5. Fig. 1. *Hypotrachyna rockii* (Zahlbr.) Hale. Fig. 2. *H. orientalis* (Hale) Hale Fig. 3. *H. microblasta* (Vain.) Hale. Fig. 4. *H. crenata* (Kurokawa) Hale. Fig. 5. *H. immaculata* (Kurokawa) Hale—scale in mm.

Specimens examined : 73·2468 Thekadi sanctuary, Kerala; 74·1031 Panhala Fort, Maharashtra; 74·3358 Talacavery, Coorg, Karnataka; 74·3390 Mercara, Coorg, Karnataka; 74·3434 Baghmandala, Coorg, Karnataka; 74·3538 Kottagiri, Tamil Nadu.

Geographical Distribution : Japan.

3. *Hypotrachyna immaculata* (Kurokawa) Hale
= *Parmelia immaculata* Kurokawa

Thallus corticolous, closely adnate to the substratum, mineral gray, 3–4 cm broad; lobes sublinear to linear, 2–2·5 mm broad; upper surface sorediate, soralia large, mostly subterminal, capitate; lower surface black to the margin, densely rhizinate; apothecia not seen.

Chemistry : Cortex K + yellow, medulla K + reddish, C –, KC + reddish, P –. Atranorine and lividic acid group present.

Specimens examined : 73·1810 Kodaikanal, Tamil Nadu; 73·2062, 73·2181 Kodaikanal—Munnar Road, Kerala; 74·3464 Mudumalmalai sanctuary, Tamil Nadu.

Geographical Distribution : South Africa, Java.

4. *Hypotrachyna microblasta* (Vain.) Hale
= *Parmelia microblasta* Vain.

Thallus saxicolous closely adnate to loosely attached, 5–6 cm broad, yellowish green in colour; lobes sublinear to linear, 1–2 mm broad; upper surface shiny, densely isidiate, isidia cylindrical, simple or coralloid branched; lower surface black to the margin, densely rhizinate; apothecia not seen.

Chemistry : Cortex K –, medulla K + red, P + orange. Usnic acid, norstictic acid, salacinic acid, galbinic acid and atranorine (traces) present.

Specimen examined : 75·46 Silver Cascade, Kodaikanal, Tamil Nadu.

Geographical Distribution : Brazil, Colombia, Hawaii Islands, Jamaica, Mauritius, Java.

5. *Hypotrachyna orientalis* (Hale) Hale
= *Parmelia orientalis* Hale

Thallus corticolous, closely adnate to the substratum, 2–4 cm broad; lobes sublinear to linear, 1–2 mm broad; upper surface isidiate, isidia cylindrical, simple or branched; lower surface black with narrow faint brown marginal zone, sp rhizinate; apothecia not seen.

Chemistry : Cortex K + yellow, medulla C + orange, P –. Atranorine, barbatic acid 4–0 dimethylbarbatic acid present.

Specimens examined : 73·519, 74·3541 camund, Tamil Nadu; 75·215 Kodaikanal, Nadu.

Geographical Distribution : Nepal, Phillip Malaya.

6. *Hypotrachyna rockii* (Zahlbr.) Hale
= *Parmelia rockii* Zahlbr.

Thallus corticolous or saxicolous, closely or loose, 4–7 cm broad; mineral gray in lobes sublinear, 1–3 mm broad; upper shiny, initially pustulate near the lobe becoming coarsely sorediate; lower surface upto the margin, densely rhizinate; apothec seen.

Chemistry : Cortex K + yellow, medull C + rose, P –. Atranorine, evernic and le acid present.

Specimens examined : 75·49, 75·53 Kod Tamil Nadu; 75·253, 75·260 Kodaikanal—M Road, Tamil Nadu.

Geographical Distribution : North, Cent South America, Japan, Indonesia; Hawaii

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---

1. Awasthi, D. D., "Catalogue of the from India, Nepal, Pakistan and *Beih. Nova Hedwigia*, 1965, 17,
2. Culberson, C. F., "Improved conditi new data for the identification of products by standardized thin-layer matographic method," *J. Chro* 1972, 72, 113.
3. Hale, M. E., "Delimitation of the genus *Hypotrachyna* (Vain.) *Phytologia*, 1974, 28, 340.

LETTERS TO THE EDITOR

FRANCK-CONDON FACTORS AND *r*-CENTROIDS OF $^3\Pi$—$X^1\Sigma$ SYSTEM OF InCl MOLECULE

THE band spectrum of InCl was studied by Patrikaln and Hochberg[1], Wehrli and Miescher[2], Youngner and Winans[3], Ashrafunnisa *et al.*[4,5] and Rao *et al.*[6]. As *r*-centroids and Franck-Condon factors play an important role in finding out the variation of electronic transition moment with internuclear distance, it is worthwhile to compute the *r*-centroids and Franck-Condon factors of the bands of $^3\Pi_{0,1} - X^1\Sigma$ sub-systems of InCl molecule. In the present calculations, the necessary constants are taken from the work of the authors[4–6].

The *r*-centroids ($r_{v',v''}$), representing the characteristic internuclear separation of a $v' \rightarrow v''$ transition in a diatomic molecular band system, has been defined by Nicholls and Jarmain[7] as

$$r_{v',v''} = \int \psi_{v'}\, r\psi_{v''}\, dr \Big/ \int \psi_{v'}\, \psi_{v''}\, dr$$

where $\psi v'$ and $\psi v''$ are the vibrational wave functions of the upper and lower states and r is the internuclear distance. In the present work, the quadratic method has been used for the evaluation of *r*-centroids for the bands of A–X and B–X systems of InCl molecule and the results are presented in Tables I and II respectively. A smooth curve has been obtained when a graph is drawn between $r_{v',v''}$ and $\lambda_{v',v''}$ as reported earlier by Nicholls and Jarmain in several cases.

As $|\, d\alpha/\alpha\,|$ is greater than 5%, the Franck-Condon factors of A–X and B–X sub-systems of InCl molecule are calculated by employing the analytical method of Fraser and Jarmain[8] with r_e-shift correction and are presented in Tables I and II along with the wavelengths of the band heads and *r*-centroids. It is from Tables I and II that the bands $v' = 4\ (v'' = 0)$) and $v'' = 5\ (v' = 0)$ could not be experimentally observed due to the negligibly small values of the Franck-Condon factors.

TABLE I

Franck-Condon factors and r-centroids of $A^3\Pi_0 - X^1\Sigma$ *system of InCl molecule*

v'	v''	0	1	2	3	4	5
0	*a*	0·565	0·301	0·099	0·027	0·006	0·001
	b	2·368	2·315	2·269	2·228	2·191	2·158
	c	3599·2	3640·5	3682·5	3726·2	3758·5	3812·5
1	*a*	0·343	0·104	0·279	0·174	0·069	0·022
	b	2·433	2·372	2·319	2·273	2·232	2·195
	c	3556·2	3596·4	3637·4	3678·98	3621·3	3764·4
2	*a*	0·082	0·382	0·000	0·166	0·194	0·110
	b	2·509	2·437	2·376	2·323	2·277	2·236
	c	3514·6	3553·9		3634·5	3575·9	3717·8
3	*a*	0·011	0·178	0·295	0·036	0·066	0·167
	b	2·602	2·513	2·441	2·378	2·327	2·281
	c	3474·5	3513·0	3552·0		3632·0	3673·0
4	*a*	0·001	0·032	0·254	0·179	0·101	0·011
	b	2·718	2·606	2·518	2·445	2·384	2·331
	c	3435·7	3473·4	3511·5	3550·2		

a—Franck-Condon factors; *b*—*r*-centroids; *c*—Wavelengths in A.U.

TABLE II

Franck-Condon factors and r-centroids of $B^3\Pi_1 - X^1\Sigma$ *system of InCl molecule*

v'	v''	0	1	2	3	4	5
0	*a*	0·586	0·291	0·092	0·024	0·006	0·001
	b	2·368	2·314	2·267	2·226	2·190	2·157
	c	3498·9	3538·0	3577·5	36·187		
1	*a*	0·333	0·128	0·284	0·165	0·063	0·019
	b	2·435	2·372	2·318	2·271	2·230	2·193
	c	3459·2		3535·3	3574·6	3614·5	3654·9
2	*a*	0·072	0·391	0·004	0·183	0·190	0·101
	b	2·516	2·349	2·375	2·321	2·275	2·234
	c	3419·6	3453·8		3532·8	3571·8	3611·4
3	*a*	0·008	0·162	0·323	0·023	0·088	0·172
	b	2·617	2·580	2·443	2·379	2·325	2·279
	c	3381·9	3418·3				3569·3
4	*a*	0·000	0·025	0·240	0·216	0·082	0·022
	b	2·752	2·621	2·524	2·447	2·383	2·329
	c		3381·1	3417·1			

a—Franck-Condon factors; *b*—*r*-centroids; *c*—Wavelengths in A.U.

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1. Patrikaln, A. and Hochberg, J., *Z. Phys.*, 1933, **86**, 214.
2. Wehrli, M. and Miescher, E., *Helv. Phys. Acta*, 1934, 7, 298.
3. Youngner, P. and Winans, J. G., *J. Mol. Spect.*, 1960, **4**, 23.
4. Ashrafunnisa, Rao, D. V. K. and Rao, P. T., *Physica*, 1974, 73, 421.
5. —, — and —, *Spect. Lett.*, 1976, **9**, 9.
6. Rao, M. L. P., Rao, D. V. K. and Rao, P. T., *Ibid.*, 1976, **9**, 107.
7. Nicholls, R. W. and Jarmain, W. R., *Proc. Phys. Soc.*, 1956, **A69**, 253.
8. Jarmain, W. R., and Fraser, P. A., *Ibid.*, 1953, A66, 1153.

RELAXATION TIMES AND ACTIVATION ENERGIES OF SUBSTITUTED TOLUENES AND THEIR DIPOLAR MIXTURE

THE present communication reports the relaxation times and activation energies of 3-fluoro-6-amino toluene, 3-fluoro-6-nitro toluene and their dipolar mixtures in dilute solutions of benzene at 3 cm microwave region. The measurements have been made a 30° and 40° C, in order to calculate free ener activation for the process of dipole orientati viscous flow. It has been concluded that the cules are relaxed predominantly by a process o all molecular rotation, and relaxation time of mixture is the average of the relaxation times two molecules.

Relaxation times τ were determined from t frequency method of Gopala Krishna[1] for solutions as discussed in an earlier paper[2]. S wave technique of Roberts and Von Hippel[3] v for measurement of dielectric constant ϵ' and factor ϵ''. Activation energies have been ca using Eyring's[4] equation.

All the substances used are of L.R. grade (Analar benzene (B.D.H.) has been distilled use. The values of relaxation time and thermo parameters are reported in Table I.

It is observed from the table that the relaxat of 3-fluoro-6-aminotoluene is smaller than t fluoro-6-nitro toluene. This is not only du smaller size of the former molecule but als the intramolecular rotation of the amino gro its bond with the ring.

It is evident from the table that the relaxa obtained for dipolar mixture in benzene is th of the two individual relaxation times of components. The experimental results sug there is no association or complex form

TABLE I

Values of relaxation times τ and thermodynamic parameters

Compounds	Temperature °K	$\tau \times 10^{12}$ sec	F_τ Kcal/mol	F_η Kcal/mol	S_τ Cal/mol	S_η Cal/mol	H_τ Cal/mol	H_η Kcal/mol
(i) 3-fluoro 6-amino toluene	293	8·02	2·26	2·90	−1·49	−1·29	1·82	2·52
	303	7·11	2·28	2·92	−1·51	−1·32	1·82	2·52
	313	6·07	2·31	2·94	−1·57	−1·34	1·82	2·52
(ii) 3-fluoro 6-nitro toluene	293	16·67	2·68	2·90	−0·92	−1·29	2·41	2·52
	303	15·12	2·74	2·92	−1·09	−1·32	2·41	2·52
	313	13·98	2·80	2·94	−1·21	−1·34	2·41	2·52
(iii) 3-fluoro-6-nitro toluene + 3-fluoro-6-amino toluene	293	12·83	2·53	2·90	−1·02	−1·29	2·23	2·52
	303	11·47	2·57	2·92	−1·12	−1·32	2·23	2·52
	313	10·29	2·61	2·94	−1·21	−1·34	2·23	2·52

where τ = Relaxation time;
F_τ = Activation energy for dipole orientation;
S_τ = Entropy of activation for dipole orientation;
H_τ = Heat of activation for dipole orientation;
F_η = Activation energy for viscous flow;
S_η = Entropy of activation for viscous flow;
H_η = Heat of activation for viscous flow.

otherwise the relaxation times would be different. A similar result has been obtained earlier by one of the authors[5]. This conclusion is in conformity with the fact that in dilute solutions, the dipole-dipole interaction is considerably reduced.

The free energies of activation, for the process of dielectric relaxation and the viscous flow increase with temperature and size of the molecules. Similar results have earlier been obtained by Smyth and collaborators[6–8] and also by the present authors[9]. This is possible because at higher temperatures the molecular collision rate increases. The entropies of activation are found to be negative indicating that the activated state is more ordered than the normal state. Further, the heats of activation and energies of activation for viscous flow are greater than those for dipole relaxation, since the former involves both rotation and translation, while the latter only rotation.

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---

1. Gopala Krishna, K. V., *Trans. Faraday Soc.*, 1957, **53**, 767.
2. Mehrotra, N. K. and Somevarshi, S. K. S., Sent for publication in the *Ind. J. Pure and Appl Phys.*, Proof No. P–2703.
3. Roberts, S. and Von Hippel, A., *J. Appl. Phys.*, 1946, **17**, 610.
4. Eyring, H., Glasstone, S. and Lailder, K. J., *The Theory of Rate Process*, McGraw-Hill Co., New York, 1941, p. 548.
5. Mehrotra, N. K. and Saxena, M. C., *Curr. Sci.*, 1966, **35**, 485.
6. Grubb, E. L. and Smyth, C. P., *J. Am. Chem. Soc.*, 1961, **83**, 4122.
7. Kalmann, O. F. and Smyth, C. P., *Ibid*, 1960, **82**, 783.
8. Heston, E. J., Heston, W. M. and Smyth, C. P., *Ibid.*, 1948, **70**, 4102.
9. Pant, D. C., Somevanshi, S. K. S. and Mehrotra, N. K., Sent for publication in *Indian Journal of Physics*.

FORMATION CONSTANTS OF Co (II), Ni (II), Cu (II), Zn (II), Cd (II), UO$_2$ (II) AND VO (II) CHELATES OF THE SCHIFF BASE DERIVED FROM PYRROLE-2-CARBOXALDEHYDE AND β-ALANINE

A SURVEY[1,2] of the literature has revealed that no work has been done on the metal-complexes of 3-(2-pyrrolideneimino)-propionic acid (H_2PP) Schiff base derived from pyrrol-2-carboxaldehyde and β-alanine. Hence the work on the potentiometric studies of its chelates with Co (II), Ni (II), Cu (II), Zn (II), Cd (II), UO_2 (II) and VO (II) was undertaken. The measurements were carried out by Calvin-Bjerrum pH-titration technique[3,4] at 25° C, 30° C and 35° C in aqueous medium (0·1 M $NaClO_4$).

Precision pH-meter type OP : 205 No. 837 with glass-calomel assembly was used. H_2PP was synthesised by a procedure similar to that reported earlier[5], m.p. 172° C, found C, 57·49; H, 5·98; N, 16·72; Calcd. for $C_8H_{10}N_2O_2$, C, 57·83; H, 6·02; N, 16·86%. All the chemicals used were either BDH or Ridiel reagents. The standard solutions of H_2PP, metal-nitrates and sodium perchlorate were prepared in doubly distilled water.

H_2PP was titrated in the absence and in the presence of the metal-ions of interest with 0·1 M sodium hydroxide solution at 25° C, 30° C and 35° C and the titration curves had the usual shapes.

In the case of H_2PP, the formation curves extend upto $\bar{n}_A \approx 2\cdot0$, indicating that two protons respectively from carboxylic and imino groups are dissociated. The mean values of dissociation constants obtained by different methods (algebraic and interpolation at half $\bar{n}_A$ values) are pK_1, 8·91, 8·85 and 8·81 and pK_2, 10·12, 10·06 and 10·01 at 25° C, 30° C and 35° C respectively.

A perusal of the pK_1 values suggest that the proton of the carboxylic group in the ligand is present as a Zwitter-ion in solution.

The formation curves for all the metal-ligand systems attain their maxima at $\bar{n} < 1\cdot0$ which indicate the formation of 1 : 1 chelates only.

The values of log K_1 obtained for Co (II), Ni (II), Cu (II), Zn (II), Cd (II), UO_2 (II) and VO (II) complexes are 8·50, 10·35, 11·95, 5·55, 4·35, 13·10 and 14·20 at 25° C, 8·55, 10·40, 11·99, 5·57, 4·37, 13·15 and 14·25 at 30° C and 8·60, 10·45, 12·05, 5·60, 4·40; 13·20 and 14·30 at 35° C respectively. The sequence of stability constants, VO (II) > UO_2 (II) > Cu (II) > Ni (II) > Co (II) > Zn (II) > Cd (II) is in agreement with the Irving-Williams rule[6].

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---

1. Cotton, F. A., *Prog. Inorg. Chem.*, 19 7, 88.
2. Perry, Christopher, L., Weber. and Ja Harold, *J. Inorg. Nucl. Chem.*, 1971, 3 1031.
3. Calvin, M. and Wilson, K' W., *J. Am. Ch Soc.*, 1945, 67, 2003.
4. Bjerrum, J., "Metal amine formation in aqu solution" (P. Haase & Sons, Copenha 1941.
5. Mehta, R. K. and Gupta, R. K., *Ind. Chem.*, 1973, 11, 56.
6. Irving, H. and Williams, R. J. P., *Na* 1948, 162, 746; *J. Chem. Soc.*, 1 p. 3192.

PYROLYTIC STUDIES ON BUFFALO MILK CASEIN

IN order to evaluate the nutritive significance of and milk products prepared under different th conditions, systematic studies on the effect of on buffalo casein at different intervals of time studied. In addition, pyrolytic studies on H treated buffalo milk casein were also invest as low concentrations of H_2O_2 have been fou preserve milk[1].

Previous studies on pyrolytic effect of cow have indicated the destruction of certain ami and the release of phosphopeptides[2] and free acids. Fujimaki *et al.*[3] observed that the r casein exhibits a decrease in biological value d partial destruction and racemisation of peptide amino acids. Osner *et al.*[4] have observed the de ration of casein above 130° C and also severe de tion of certain amino acids at elevated tempera

Buffalo casein from fresh buffalo milk and cas from H_2O_2 treated buffalo milk were isolated by electric precipitation technique[5].

Hundred mg each of the (i) fresh casein (unhe (ii) heated casein; (iii) H_2O_2 treated buffalo casein, and (iv) H_2O_2 treated and heated buffal casein were hydrolysed using 6N HCl at 120° C.

The hydrolysates were spotted on Whatman filter-paper and irrigated with *n*-butanol, water, acid (4:1:1) by employing ascending paper chro graphic technique. The separated amino acids identified by treatment with ninhydrin in 95% aq acetone and quantitatively estimated using de meter. For overlapping amino acids such as ly histidine, serine, glycine, aspartic acid, etc., the dimensional development technique was emp using phenol-water in the ratio 4:1 as the second sol Similar experiments were carried out with the

TABLE I

Percentage loss of various amino acids in Buffalo milk casein

(Average values of three samples)

Amino acids	Average % loss of heated B.M.C.* for 1 h	Average % loss of heated B.M.C.* for 24 h	Average % loss of H_2O_2 treated and heated B.M.C.* for 1 h			
			concentration of H_2O_2			
			0·02%	0·04%	0·08%	0·10%
1. Lysine	2·8	67·2	13·2	27·0	54·1	67·6
2. Histidine	2·7	65·3	13·4	26·8	53·4	66·8
3. Arginine	2·3	56·1	12·9	22·5	42·4	56·1
4. Serine	2·1	50·4	13·6	23·4	38·4	49·2
5. Glycine	1·8	42·7	8·6	17·4	34·1	42·8
6. Aspartic acid	2·4	57·6	12·6	24·4	50·8	62·0
7. Threonine	2·6	62·6	17·1	26·2	48·2	59·5
8. Alanine	2·1	50·6	12·7	17·5	35·1	50·4
9. Tyrosine	2·4	58·8	12·1	24·2	49·2	60·4
10. Valine	2·9	69·6	13·6	26·2	54·4	69·4
11. Methionine	2·6	62·4	13·7	26·8	52·4	68·2
12. Phenylalanine	2·3	56·6	13·8	22·9	44·8	53·6
13. Leucines	1·7	40·8	8·2	16·4	33·1	40·6

B.M.C.*—Buffalo milk casein.

samples of the hydrolysates prepared under identical conditions. The developed chromatogram was used for the estimation of amino acids densitometrically. The results are given in Table 1.

RESULTS AND DISCUSSION

From the above results it is clearly indicated that thermally treated casein for a period of 1 h at 110° C has undergone a small decrease (2·5%) in most of the amino acids. When casein is heated for 24 h under identical conditions the loss is over 50%.

In the case of casein isolated from milk treated with H_2O_2, it is observed that even at a very low concentration of 0·02%, there is over 10% loss of amino acids on heating for an hour at 110° C. At higher concentrations of H_2O_2 (0·1% by wt/v) there is a marked decrease of amino acids over 60%.

H_2O_2 treated casein on pyrolysis at 110° C readily turns brown releasing gaseous products. So the H_2O_2 treated sample (0·02 wt/v) should not be heated for over 30 min. at 110°C.

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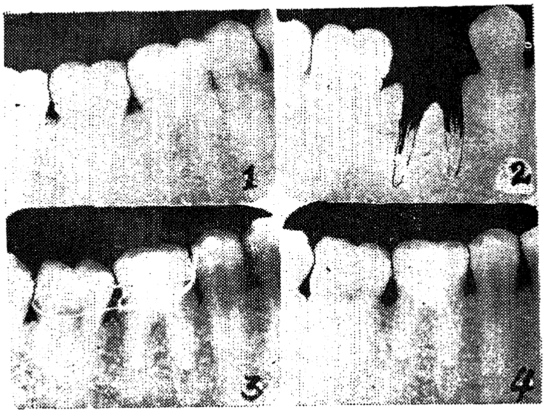
---

1. Srinivasan, A. and Gopalan, S., *Curr. Sci.*, 1975, 44 (12), 423.
2. Lorient, D. and Alais, C., *Int. Dairy Cong.*, 1970, 1E, 54.
3. Fujimaki, M., Kato, H. and Hayase, F., *Agri. Biol. Chem.*, 1972, 36 (3), 416.
4. Osner, R. C. and Johnson, R. M., *J. Food. Tech.*, 1974, 9 (3), 301.
5. Ganguly, N. C., Prabhakaran, R. J. V. and Iya, K. K., *J. Dairy Sci.*, 1964, 47, 13.

ON THE REPLANTATION OF HUMAN MOLARS

MODERN operative dental practice involves *replantation* of the traumatically displaced teeth as well as *intentional replantation*[1, 2], attempting to treat and retain the permanent teeth affected with caries—in such cases where *in vivo* endodontic surgery is not feasible. *In vitro* endodontic treatments are carried out in the shortest possible time thus maintaining the viability of the supporting structures of the extracted teeth[3]. Further, the limitation of endodontic therapy of the carious teeth, extent of coronal destruction and periodontal involvement and, oral hygiene are some of the important criteria to be considered while attempting an *intentional replantation* towards a conservative management. Present study was undertaken with reference to the adult molars and to check the viability of the periodontal ligaments when excised, though a few attempts have been made in relation to the replantation of permanent anterior teeth[4-5].

Root canals of carefully extracted molars of ten adult humans were treated *in vitro* (Figs. 1 and 2) with ready-made gutta percha points and zinc oxide-eugenol cement. Zinc-free silver amalgam fillings were provided at the apical region, following the removal of 2–3 mm of the apical portion of the roots. Crowns of the teeth were restored by permanent fillings and the occlusal surfaces of the teeth were ground to prevent contact with the opposing teeth. Effective reinsertion in the socket was made with wiring with neighbouring teeth (Figs. 3 and 4), for a period of 6–8 weeks.



FIGS. 1–4. Successive stages of replantation procedure of human molar.

Though the exact biological processes involved in the successful replantation of intentionally or accidentally displaced teeth are not known, the procedure is gaining importance owing to its aesthetic value especially in teeth with calcified root canals where radiographic evidence of apical pathology is not exhibited. Extensive root resorption has been reported[6] in the replantation studies of the anterior teeth of children where the ligaments and the supporting tissue were suggested to be in embryonic stage. Present study confirms the periodontal membrane and the exposed adult teeth portions being viable at least for a period of 20–30 min., as in, 90% of the analysed samples no resorption occurred for a period of 4–5 years. In spite of the indication of the post-operative regeneration of the periodontal ligament being similar to the periosteum of the bone grafts[6], the successful replantation of the adult molars strongly favours experimental furtherance in terms of physiological stability or loss, and histology of regenerating supportive tissue, over extended periods.

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---

1. Grossman, L. I. and Chacker, F., *Trans. Fourt Internat. Conf. Endodont.*, 1968, 127.
2. Deeb, E., *Ibid.*, 1968, 147.
3. Flanagen, V. D. and Myers, H. I., *Oral Surg. Med. Path.*, 1958, **11**, 1179.
4. Rajan, B. P., *Internat. Coll. Dent. News Lett*, 1973, 10.
5. Parameswaran, A. and Ramesh, R., *Dental Dia logue*, 1975, **2** (3), 25.
6. Monsour, F. N. T., *Aust. Dent. J.*, 1970, **15**, 3

IDENTITY OF THE PATHOGEN CAUSING ERGOT OF PEARL MILLET IN INDIA

ERGOT contamination of pearl millet (*Pennise typhoideum*) is a serious problem in India, since co sumption of ergoty pearl millet leads to disease humans characterized by nausea, vomiting, giddi and prolonged sleepiness[1].

The pathogen causing ergot of pearl millet in In was identified by Shinde and Bhide[2] as *Clavic microcephala*. Loveless[3] designated the path causing ergot of pearl millet in Rhodesia as *Clavi fusiformis*. An attempt was, therefore, made establish the correct identity of the pathogen of millet in India based on morphological and che taxonomic criteria.

A number of samples of ergot of pearl millet collected from various parts of India and exami The sclerotia were obpyriform or irregularly sh up to 0·7 mm long and up to 0·4 mm wide, gene slightly curved with maximum width at the base usually narrowing towards the tip. The conidia hyaline, fusiform to broadly falcate, and 28·0–4 wide. Comparison of the Indian material with type material of *Claviceps fusiformis* established the two were identical.

A study of the alkaloids in the honeydew s after extraction and quantitation by the method cribed earlier[4] for sclerotial stage, showed tha gm of the material contained about 5 mg of the loid. The alkaloid consisted of agroclavine, e clavine, chanoclavine, setoclavine and pennicl The alkaloid profile was found to be similar to seen in the sclerotial stage. However, quanti in the sclerotial stage the alkaloid content was to be ten fold higher than that in the honeydew The pathogen which causes ergot of pearl millet pro the clavine group of alkaloids both in sclerotial as well as the honeydew stages, whereas, the pathogen *C. purpurea* responsible for ergot of rye produces the ergotoxine-ergotamine group of alkaloids. Thus based on morphological and chemotaxonomic characters it is clear the pathogen that leads to ergot of pearl millet in India is *Claviceps fusiformis* Loveless.

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---

1. Krishnamachari, K. A. V. R. and Bhat, R. V. *Ind. J. Med. Res.*, 1976, 64, 1624.
2. Shinde, P. A. and Bhide, V. P., *Curr. Sci.*, 1958, 27, 499.
3. Loveless, A. R., *Trans. Brit. Mycol. Soc.*, 1967, 50, 15.
4. Bhat, R. V., Roy,. D. N. and Tulpule, P. G., *Toxicol. Appl. Pharmacol.*, 1976, 36, 11.

CHLORAMPHENICOL RESISTANCE IN GENUS *BACILLUS*

CHLORAMPHENICOL resistant bacteria such as *Escherichia coli*[1, 2, 3]; *Pseudomonas aeruginosa*[1]; *Pseudomonas fluorescens*[4]; *Proteus mirabilis*[1] and *Staphylococcus aureus*[5,6] have been reported and the mechanism of the resistance has been studied. S. Osawa isolated a number of chloramphenicol resistant mutants from *Bacillus subtilis ATCC* 6633 which are resistant to 5 μg of chloraamphenicol[7]. However, there has been no report on naturally occurring strain of *Bacillus* which is resistant to chloramphenicol. The author has isolated several chloramphenicol-resistant strains of *Bacillus* from soil. One of the isolates, showing similar bacteriological properties as *Bacillus megaterium*, was used throughout the experiment (Strain 7). It was gram positive spore forming bacilli, giving typical large, round, opaque whitish colony on nutrient agar, produces acid only from mannitol, sucrose, glucose, arabinose and xylose. Lactose was variable. Isolates were identified following the scheme of *Bergey's Manual of Determinative Bacteriology*, 7th Ed. and *A Guide Book to the Identificaton* of Genera *of Bacteria* (V.B.D. Skerman, 1967). *Bacillus megaterium* strain 7, could grow in the presence of 10 μg/ml and was trained to grow by subculture in increasing concentrations of chloramphenicol (50 μg/ml chloramphenicol). *Bacillus megaterium KM* and *Bacillus cereus* T, used as standard, could not grow at the concentration of 10 μg/ml. In order to measure chloraamphenicol activity, the cells of chloramphenicol-sensitive and resistant strains of *Bacillusmegaterium* were incubated in the nutrient broth containing 250 and 500 μg/ml of chloramphenicol for 0, 8 and 20 h at 37° C with shaking. [Nutrient broth consisted of meat extract (Kyokuto Seiyaku Co., Tokyo) 10·0 g.; polypeptone (Daigo Eiyo Kahaku Co., Osaka) 10·0 g.; sodium chloride 5·0 g; deionized water 1000 ml pH 7·3]. The amount of chloramphenicol in the medium was bio-assayed using the cup-plate method. Only a weak inactivation was observed when the resistant strain was used. To increase the sensitivity of the test, the cells of either the resistant strain (*Bacillus megaterium* strain 7) or the sensitive strain (*Bacillus megaterium KM* obtained from the collection of this laboratory) was incubated with ^{14}C– chloraamphenicol in nutrient broth and the degradation products of ^{14}C-chloramphenicol in the supernatant were analyzed by chromatography and autoradiography. Degradation products of ^{14}C-chloramphenicol were detected only when the resistant strain was used (uninduced condition). The amounts of degradation products were markedly increased when the cells of the resistant strain, which were pregrown in the presence of 10 μg/ml of chloramphenicol, were incubated for 20 h with ^{14}C-chloramphenicol (induced condition). No degradation products, however, were detected when the cells of *Bacillus megaterium KM* were incubated with ^{14}C-chloramphenicol under similar uninduced or induced conditions. Degradation of ^{14}-C-chloramphenicol was also demonstrated when the cell-free extract of *Bacillus megaterium* strain 7 was incubated with ^{14}C chloramphenicol, acetyl-CoA and Mg^{++} ion, while no degradation was observed with the cell-free extract of *Bacillus megaterium KM*. Thus, chloramphenicol decomposing enzyme in *Bacillus megaterium* strain 7 may partly be responsible for the chloramphenicol resistance of this organism. Further work on the study of mechanism of the resistance is in progress.

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1. Okamoto, S. and Suzuki, Y. *et al.*, *J. Bact.*, 1967, **94**, 1616.
2. Sompolinsky, D. and Samra, Z., *J. Gen. Microbiol.*, 1968, **50**, 55.
3. Shaw, W. V., *J. Biol. Chem.*, 1967, **242**, 687.
4. Donn J. Kushner, *Arch. Bicohem. Biophys.*, 1955, **58**, 347.
5. Kono, M., Ogawa, K. and Mitsuhashi, S., *J. Bact.*, 1968, **95**, 886.
6. Shaw, W. V. and Brodsky, R. F., *Ibid.*, 1968, **95**, 28.
7. Osawa, S., Takata, R. Tanaka, K. and Tamaki, M., *Molec. Gen. Genet.*, 1973, **127**, 163.
8. Freed, R. S., Murray, E. G. D. and Smith, N. R., *Bergey's Manual of Determinative Bacteriology*, The Williams and Wilkins Company, Baltimore, U.S.A., 1957.
9. Skerman, V. B. D., *A Guide Book to the Identification of Genera of Bacteria*, 1967.

FUNCTIONAL REGRESSIONS IN FISHERY RESEARCH

IN linear regression situations arising in fishery biology, Ricker[1] has recommended the use of a functional regression, when both the variates are subject to error of measurement or inherent variability or both. In such cases the regression line is obtained by finding the line which minimizes the sum of the products of the vertical and horizontal distances of each point from the line. Ricker refers to the estimate of the regression coefficient thus obtained as the 'GM regression'.

If, instead of sum of products, the sum of squares of both the horizontal and vertical distances of each point from the line is considered, another line with slope very close to the GM regression is obtained. Let the regression line of y on x be

$$y = a + bx. \tag{1}$$

If P be any point (x,y) such that PM and PN are the vertical and horizontal distances from the line $(x, a + bx)$ and $[(y - a/b, y)]$ are the coordinates of M and N respectively. Obviously, the distances PM and PN are $(y - a - bx)$ and $[(y - a/b) - x]$, Minimizing $PM^2 + PN^2$ is same as minimizing MN^2, MN being the hypotenuse of the right-angled triangle PMN. Minimization of MN allows the line to shift itself towards the point, which is ideally required.

Considering all such n points as P, the quantity

$$\sum_{i=1}^{n} \left\{ (y_i - a - bx_i)^2 + \left(\frac{y_i - a}{b} - x_i\right)^2 \right\} \tag{2}$$

is to be minimized with respect to a and b: Differentiating (2) with respect to a and equating to zero gives, after simplification,

$$\sum_{i=1}^{n} y_i = na + b \sum_{i=1}^{n} x_i \tag{3}$$

Similarly differentiation with respect to b leads to the simplified form

$$b(b^2 - 1) \sum_{i=1}^{n} y_i x_i - ab(b^2 - 1) \sum_{i=1}^{n} x_i - b^4 \sum_{i=1}^{n} x_i^2 + \sum_{i=1}^{n} (y_i - a)^2 = 0. \tag{4}$$

If from (3) substitutions are made for a, (4) simplifies to

$$b^4 \left[\sum_{i=1}^{n} x_i^2 - \frac{\left(\sum_{i=1}^{n} x_i\right)^2}{n} \right] - b^3 \left[\sum_{i=1}^{n} x_i y_i - \frac{\sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{n} \right] + b \left[\sum_{i=1}^{n} x_i y_i - \frac{\sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{n} \right] - \left[\sum_{i=1}^{n} y_i^2 - \frac{\left(\sum_{i=1}^{n} y_i\right)^2}{n} \right] = 0,$$

which may be replaced by

$$S_x^2 b^4 - S_{xy} b^3 + S_{xy} b - S_y^2 = 0, \qquad (5$$

where S_x^2, S_y^2, S_{xy} are respectively the variances c x and y and the covariance between them.

Employing the method of iteration, starting wi the estimate of GM regression as a trial value wi lead to a solution of (5) which being the slope of t line under consideration.

In the case of GM regression since the estima involves only the standard deviations of the variabl the pairing up of x and y has no effect. But, sin the values come from a bivariate distribution, t association of the variables has to be given due con deration when finding the functional relationsh Here, this is guaranteed by the involvement of covarian in the esimating equation (5).

The regression of x on y is also the same, but i slope will be $1/b$ since the axes are reversed. T is obtained by interchanging the expressions variances of x and y in the esimating equation and solving.

The variance of this regression is same as t of the corresponding GM regression.

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1. Ricker, W. E., *J. Fish. Res. Board Can.*, 1 30, 409.

EFFECT OF ACTINOMYCIN D ON CHIASMA FREQUENCY IN *CHRYSOCORIS PURPUREUS* (WESTW.) PENTATOMIDAE-HETEROPTERA

IT has been shown that actinomycin D (AD) binds to DNA and inhibits the synthesis of RNA[1]. The drug is capable of inducing mitotic inhibition and structural changes in chinese hamster metaphase chromosomes *in vitro*[2]. Further the effect of AD on chiasma frequency has already been described in *Vicia faba* and *Schistocerca gregaria*[3,4]. The chromosomes of most eukaryotes have a localized kinetochore which presents as a primary constriction by light microscopy. The pentatomid bug *Chrysocoris purpureus* represents holokinetic nature of chromosomes. The relationship between kinetochore structure and resistance to radiations has been reported[5,6]. The present study has been undertaken to provide information on chiasma frequency of *C. purpureus*.

Adult males were each injected abdominally with either 0·01 ml. of aqueous actinomycin D (10 μg/ml.) or with 0·01 ml. of distilled water. The insects were released on *Croton sparsiflorus* in cages. Control and treated insects were removed at random after specified intervals. The testes were dissected out in insect saline and fixed in Acetic alcohol (1 : 3) and processed as haematoxylin squashes.

The large number of meiotic cells are active in synthesising nucleic acids and proteins during gametogenesis. This is essential for the formation of chiasma preferentially between chromosomes that were structurally identical. A perusal of Table I would show the increase of chiasma frequency as compared with that in control. The observed increase in the frequency of chiasma in holokinetic chromosomes of *C. purpureus* is comparable with *S. gregaria*. Chromosomes with holokinetic and localized kinetochore exibit identical response with AD. It is clear from these studies that the biochemical interactions of AD with chromosomes are similar in both cases. The probable action and mechanism of the drug has been reported earlier with special reference to chiasma frequency[4]. Probably AD might play a role in activating kinetochores and chromosomes in favour of chiasma formation.

TABLE I

*Mean Chiasma frequencies for the Different Hours of Treatment (AD)**

Hours of treatment	Treated	Control
6	6·60±0·84	6·22±0·78
12	7·20±0·92	6·10±0·86
24	7·40±0·78	6·24±0·88
48	7·24±0·80	6·20±0·89
Total Mean	7·11±0·83	6·19±0·85

* Based on 25 diplotene—diakinesis.

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---

1. Cavalieri, L. F. and Nemchin, R. G., *Biochim. Biophys. Acta*, 1964, 87, 641.
2. Arrighi, F. E. and HSu, T. C., *Exp. Cell. Res.*, 1965, 39, 305.
3. Sharma, R. P. and Singh, D., *Chromosoma*, 1968, 24, 309.
4. Craig-Cameran, T. A., *Ibid.*, 1970, 30, 169.
5. North, D. T. and Holt, G. G., *J. Econ. Ent.*, 1968, 61, 928.
6. Lachance, L. E., Degrugillier, M., Leverich, A. P., *Chromosoma*, 1970, 29, 20.

DIETARY INFLUENCE ON RESPIRATION IN SOME CALLIPHORID AND SARCOPHAGID FLIES (DIPTERA, INSECTA)

THOUGH the importance of protein injestion has been studied in depth[1], very few studies have been conducted to probe the physiological reasoning of injestion of sugar content[2]. Ecologically, this is most important as the major carbohydrate material available to flies in the field is nectar and honey[3]. The present note deals with the comparative effect of sugar deficient diet on oxygen consumption in males and females of *Lucilia cuprina* Weidemann, *Chrysomia megacephala* Fabricius, *Chrysomia rufifacies* Macquart and *Sarcophaga ruficornis* Fabricius.

Flies were provided with a balanced diet of meat pieces, granulated glucose and water in the normal rearing set-up. In experiments lacking sugar, the diet consisted of meat pieces and water. Flies were starved for six hours prior to experimentation. Mild etherisation was used in handling the flies. Wet weight determinations were made prior to measurements of respiratory rates. Individual flies were introduced in manometric flasks for measurement of respiratory rates. Determinations of O_2 consumption were made at the same time each day at 27° C with Warburgh respirometer. Flies (1, 4, and 7 day old) were selected to cover all the major developmental features of the reproductive cycle in blow flies[4].

TABLE I

Oxygen consumption at 27°C

Species	Sex	Mean of $\mu l \cdot O_2$ uptake/mg/hr in male and female blowflies provided with sugar (A), sugar lacking diet (B), corresponding difference (C) and percentage decrease (D).*											
		1 day old				4 days old				7 days old			
		A	B	C	D	A	B	C	D	A	B	C	D
L. cuprina	♂	2·27	1·79	0·48	21·00	2·43	1·88	0·55	22·70	2·69	2·10	0·59	22·00
	♀	2·56	2·48	0·08	3·20	3·78	3·67	0·11	2·90	5·32	5·17	0·15	2·80
C. megacephala	♂	1·76	1·28	0·48	27·10	1·80	1·30	0·50	28·00	2·16	1·57	0·59	27·30
	♀	1·91	1·82	0·08	4·60	3·15	3·01	0·14	4·50	4·92	4·73	0·19	4·00
C. rufifacies	♂	1·44	0·99	0·42	30·00	1·53	1·07	0·46	30·30	1·84	1·26	0·58	31·70
	♀	1·59	1·51	0·08	5·20	2·72	2·57	0·15	5·50	4·64	4·38	0·26	5·60
S. ruficornis	♂	1·12	0·68	0·44	39·20	1·26	0·75	0·51	40·80	1·56	0·97	0·59	38·00
	♀	1·43	1·32	0·11	7·80	2·38	2·21	0·17	7·40	4·12	3·86	0·26	6·30

* Average for three determinations at each time stage.

Table I gives the mean percentage decrease in O_2 uptake of male and female blowflies provided with sugar deficient diet. The fall in the male species is very significant compared to the decrease in O_2 uptake of the females.

A great variation in oxygen consumption of adult flies of the above species is known to exist[4]. It was also noted that females in general utilise more oxygen than males on μl/mg basis. Metabolic rate per unit weight in different species was found to be in the order of *L. cuprina* > *C. megacephala* > *C. rufifacies* > *S. ruficornis*. Complete reversal of this order as a response to sugar deficient diet, expressed in terms of $\mu l\ O_2$ consumed/mg/hr is a noteworthy feature of the present studies. Fall in oxygen uptake per unit weight in different species was found to be in the order of *S. ruficornis* > *C. rufifacies* > *C. megacephala* > *L. cuprina*.

The reasons for such a physiological expression perhaps lie with the eco-physiological demands imposed on these blowflies. Proteinaceous matter is known to accelerate the reproductive development and egg laying in blowfly females. Thus, in females, the protein hunger is directly associated to the function of species survival. As yet, no physiological explanation has been offered for the uptake of proteins by male blowflies[2]. The primary role of the males is to inseminate the virgin females for which it does not seem to depend on protein diet.

The reported "protein hunger" and the associated higher metabolic rate in the absence of protein diet is more in females, but surprisingly, much less in males of Calliphoridae[1,5]. The sudden fall in O_2 uptake of males in the absence of any sugar supply reveals the necessity of a carbohydrate substrate for males. This further indicates the presence of different dietary demands of males and females and confirms the drive component theory suggested by Calabrese and Stoffolano[1]. In addition, the pattern of respiration emerged as a response to sugar deficient diet helps in understanding the inherent properties of resistance of blowflies to inimical conditions. Parameters, *viz.*, body size, body weight, growth rate, total and tissue water content have satisfactorily explained the $\mu l/O_2$ uptake/mg/hr in selected Calliphoridae[4]. Perhaps the same parameters could be employed to explain the resulting order of resistance, *viz.*, *L. cuprina* > *C. megacephala* > *C. rufifacies* > *S. ruficornis*. The validity of these results in terms of survival fitness of species will be discussed elsewhere.

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1. Calabrese, E. J. and Stoffolano, J. G., *J. Insect Physiol.*, 1974, **20**, 383.
2. Kitchling, R. L. and Roberts, J. A., *Bull. Ent. Res.*, 1974, **64**, 81.
3. White, R. S., Aubertin, D. and Smart, J., *The Fauna of British India*, Vol. 6, London, Taylor and Francis Ltd., 1940.
4. Khole, V., *First All India Symposium on Comparative Physiology*, *Aurangabad*, 1975, p. 14.
5. Dethier, V. G., *Biol. Bull. Woods Hole*, 1961, **121**, 456.

CYTOCHEMICAL EVIDENCE FOR THE PRESENCE OF STRONG ACID GROUPS OF POLYSACCHARIDE IN A CILIATE PROTOZOA, *CONCHOPHTHIRIUS LAMELLIDENS AMICRONUCLEATUM*

POLYSACCHARIDE of mammals have been classified into acidic and basic groups according to their capacity to bind the basic dye, *i.e.*, methylene blue[2] at various pH[1]. Presence of weak acidg roups has been reported in mammalian polysaccharide[2] and in the polysaccharide of a ciliate protozoan *Tetrahymena pyriformis* (Strain W)[3]. As a part of a detailed cytochemical study on this ciliate, the study of methylene blue staining was carried out to assess the quality and characteristics of the polysaccharide.

Conchophthirius lamellidens amicronucleatum is a commensal ciliate in the mantle cavity and on the gills of the common fresh water mussel, *Lamellidens marginalis*[1]. These mussels were collected from Moosi River in Hyderabad, and dissected immediately. The mantle cavity and gills were washed and the ciliates were picked up with a fine pipette, air dried on slides and fixed in 10% neutral formalin. Solution of methylene blue B (5 mM) was prepared using veronal-actate buffer ranging in pH from 3·0 to 9·0 and the slides were immersed in it for 24 hours.

Capacity to bind methylene blue was demonstrated over a wide range of pH values. Binding of the dye was intense at the pH levels tried. The macronucleus, ectoplasm and basal granules were unstained, the peristomal region and the anterior thigmotactic region had a few bluish granules but the posterior endoplasm was intensity blue. The details are given in Table I.

The mammalian polysaccharide gives maximum staining with methylene blue at an alkaline pH and as the acidity is increased the dye uptake is decreased or absent[2]. In *Tetrahymena pyriformis* (Strain W) binding of methylene blue was very intense at pH levels 9·0, 8·0, 7·0, less at pH 6·0 and 5·0 and absent at pH 4·0 and below[3]. But in the present study the binding capacity of methylene blue in this commensal ciliate protozoan is unaltered as the acidity is increased. It is assumed that acid polysaccharide exists in this organism so that dissociation of acid groups is not suppressed at pH 4·0, 3·5 and 3·0.

TABLE I

Specific areas of methylene blue staining

	pH 9·0	pH 8·0	pH 7·0	pH 6·0	pH 5·0	pH 4·0	pH 3·5	pH 3·0
Macronucleus	—	—	—	—	—	—	—	—
Basal granules	—	—	—	—	—	—	—	—
Ectoplasm	—	—	—	—	—	—	—	—
Peristomal region	+	+	+	+	+	+	+	+
Anterior thigmotactic region	+	+	+	+	+	+	+	+
Region surrounding the macronucleus	++	++	++	++	++	++	++	++
Posterior endoplasm	+++	+++	+++	+++	+++	+++	+++	+++

— Negative; + Positive; ++ Strong; +++ Intense.

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1. Chakravarti, M. M., Chhaya Mitra and Ray, H. N., *Proc. Zool. Soc.*, Calcutta, 1959 (1960), **12**, 41.
2. Dempsey, E. W., Bunting, H., Singer, M. and Wislocki, G. B., *Anat. Rec.*, 1947, 98, 417.
3. Koehler, L. D. and Ferrel, R. A., *J. Morph.*, 1964, **14**, 209.
4. Pearse, A. G. E., *Histochemistry, Theoretical and Applied*, J. and A. Churchil, London, pp. 760.

ROTIFERS AS INDICATORS OF POLLUTION

DURING the course of studies on rotifers in different samples of water around Visakhapatnam, Andhra Pradesh, an interesting observation was made in harbour backwaters. A particular species of Rotifera *Brachionus plicatilis* Muller, was observed to be present only in certain periods of the year.

The area investigated is a wide shallow brackish water environment surrounded by small mangrove plants. It is highly polluted and frequently subjected to sewage and urban effluents along with the industrial wastes.

During the period from March, 1975 to February 1976, regular weekly samples of plankton were collected from two points in the above locality. The quantitative study of the rotifer, *Brachionus plicatilis*, revealed that there were two peak periods of abundance (Table I) one in summer (June) and the other in winter (January). According to George[1], Rotifers showed a summer maximum in tropical waters. But in the present observation in addition to the summer peak, *B. plicatilis* showed another peak in winter.

TABLE I

Months	Temperature °C	pH	Dissolved oxygen (Mg/L)	BOD (Mg/L)	Salinity	Species of Rotifera recorded	Organisms/liter
March 1975	26·5	8·8	4·2	10·2	9·45	*B. plicatilis*	18
April	27·5	8·5	3·7	24·3	16·11	*B. plicatilis*	98
May	29·0	8·0	1·68	26·4	17·77	*B. plicatilis*	172
June	30·5	7·5	0·82	32·0	11·33	*B. plicatilis*	1858
July	25·5	8·5	3·8	15·0	10·52	Nil	..
August	24·0	8·8	6·2	8·8	2·25	*K. tropica*	8
						R. rotatoria	12
September	24·5	8·5	7·1	6·8	3·32	Nil	..
October	23·5	9·0	6·2	10·6	3·66	Nil	..
November	24·5	8·8	3·2	12·0	8·81	Nil	..
December	22·5	8·5	2·5	14·6	6·91	Nil	..
January 1976	23·5	7·8	0·95	36·8	7·07	*B. plicatilis*	2642
February	24·5	8·0	2·5	26·0	10·55	*B. plicatilis*	266

Details of chemical analysis are given in the table, from which is is clear that the abundance of this species is mainly controlled by pH and dissolved oxygen content. High values of B.O.D. were recorded during the months of April, May, June, January and February. This speices showed a wide range of salinity tolerance 7·1–17·8%.

A few numbers of *Keratella tropica* and *Rotaria rotatoria* were also found during the period of August in the same locality, when the salinity was very low (2·25%) while they were completely absent during the remaining part of the year. But the observations from the other samples of water in Visakhapatnam showed the presence of these two species in abundance in highly polluted fresh water environments like ponds and swamps. Arora[2] reported species of *Polyarthra* and *R. rotatoria* in polluted waters of Nagpur. From the present observation, it is clear that these two species *K. tropica* and *R. rotatoria* are not able to tolerate the varying salinity conditions.

According to Ahlstrom[3], *B. plicatilis* can be regarded as an indicator of high alkalinity as this species inhabits only brackish waters. But from the foregoing account it may be concluded that the presence of this species (*B. plicatilis*) may indicate the polluted nature of the brackish water environment and its occurrence in waters having low alkalinity. Further, the absence of this species in fresh water bodies of Visakhapatnam supports the above conclusion.

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---

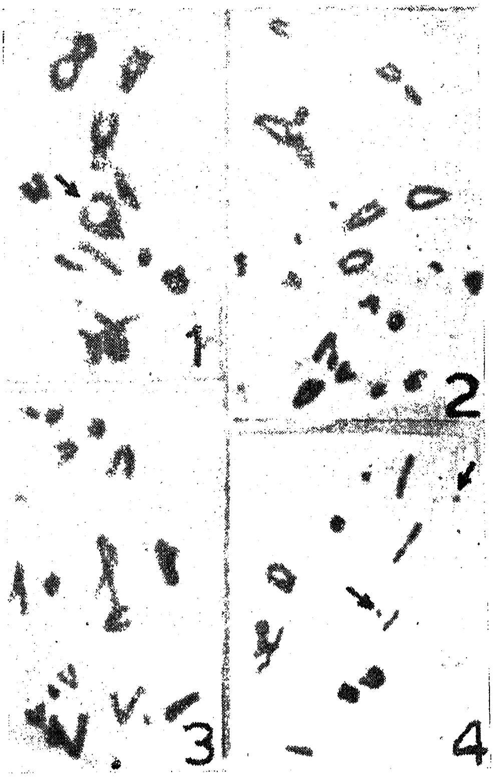
1. George, M. G., *Curr. Sci.*, 1961, 30, 268.
2. Arora, H. C., *CPHERI Bull.*, 3, 1961, 4 24.
3. Ahlstom, E. H., *Bull. Amer. Mus. Nat. Hist.*, 1940, 77, 143.

CCC INDUCED MEIOTIC INSTABILITY IN GRASSHOPPER SPERMATOCYTES

WANTON use of fungicides, insecticides and herbicides in modern agriculture has rightly raised the question of their possible adverse effects on the ecosystem at all levels: cell, individual and population. It has been reported that routine application of herbicides to fields leads to genetic and morphologic changes which contribute to the instability in the seed stocks[1,2]. In the present paper the authors have described the effects of the herbicide, chloramquat chloride, commercially known as CCC, on the spermatocytes of an acridid grasshopper *Oxya velox*.

The grasshoppers were injected with 0·5% of the chemical at a dose of 0·05 ml/individual and their testes were fixed at different intervals of time. Individuals injected with an equal dose of distilled water served as the control series.

The treated series on comparison with the control showed a much greater number of abnormal spermatocytes. The frequency of such abnormal cells was highest at 12 hours of fixation (10·6%) after which there was a decline (Table I). However, the over-all nature of the abnormality was the same regardless of the period of treatment. Out of 1788 spermatocytes examined there were only 4 cells with distinct breaks (Fig. 1) while the number of disturbed anaphases was observed in as many as 65 cells. In most of such plates, the chromosomes had failed to seggregate properly (Fig. 2) and in a few cases a number of laggards were seen (Fig. 3). A good number of metaphase I plates also disclosed the presence of two or more univalents which had resulted from the smallest pair(s). Judging from their disposition in the plates it appeared that these univalents had undergone an early separation (Fig. 4).



FIGS. 1–4

TABLE I

Frequency distribution of abnormal cells at different hours of fixation

Hour of fixation	Total No. of cells observed	Dipl.	Diak.	Met. I	Met. II	Ana. I	Ana. II	%
15 minutes	115	..	..	..	..	..	1	0·87
30 minutes	144	..	..	1	..	..	1	1·38
1 hour	110	..	..	..	..	..	2	1·81
2 hours	225	..	2	..	13	..	3	8·00
4 hours	248	..	2	2	14	..	1	7·66
8 hours	312	2	2	6	11	..	4	8·01
12 hours	179	2	2	1	..	14	..	10·61
24 hours	346	7	2	4	11	..	3	7·80
48 hours	109	1	..	1	..	5	1	7·34
Total	1788	12	10	15	49	19	16	6·78

Except for the effect of CCC on the rate of mutation in bacteria and fruit flies[3], no systematic work on its effects on other biological systems, and on the dividing cells in particular, has been, so far as known to us, undertaken. We find that CCC, unlike many of the herbicides and pesticides[4-7], does not have much adverse effect on the structural integrity of the chromosomes as evinced by the absence of stickiness, fragmentation, bridges, etc. Almost similar results have been reported in the meiotic cells of sorghum and other plant materials following the application of Atrazin, a systemic herbicide[8]. The exact mechanism by which these chemicals bring about the cytological damages is not yet clear. However, in the absence of any marked differential effects on the chromosome structure *per se*, it is probable that these chemicals disturb the physiological/biochemical systems of the cells which eventually interfere with the spindle organization.

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---

1. Unurau, J. and Larter, E. N., *Can. J. Bot.*, 1952, 30, 22.
2. Suneson, C. A., *Agron. J.*, 1960, 52, 120.
3. Kramers, P., Ada, G. N., Knapp; G. A. C. and Vood, C. E., *Mutation Res.*, 1975, 31 (1), 55.
4. Amer, S. M. and Farah, O. R., *Cytologia*, 1974, 39, 507.
5. — and —, *Ibid.*, 1975, 40, 21.
6. — and Ali, E. M., *Ibid.*, 1974, 39, 633.
7. Wuu, K. D. and Grant, W. F., *Ibid.*, 1967, 32, 31.
8. Lee, Rao, G. M., Barnett, F. L. and Liang, G. H., *Ibid.*, 1974, 39, 697.

SEXING THE PUPAE OF GRAM CATERPILLAR, *HELIOTHIS ARMIGERA* Hbn. (LEPIDOPTERA: NOCTUIDAE) IN RELATION TO CERTAIN MORPHOMETRIC CHARACTERS

SEXING insects in their larval and pupal stages can be advantageously used in studies like sex attractants, chemosterilants and male sterile technique. The position of genital opening is more often used to distinguish sex in pupae than other characters[1–3].

When the gram caterpillar, *Heliothis armigera*, was mass cultured, initially on the leaves of Bengal gram up to the third instar, and then individually on soaked Bengal gram seeds, several external morphological differences between the sexes of the pupae were noticed. The genital opening in the male was located on the posterior region of the 9th abdominal segment and was flanked by a pair of pads. The female pupae had their gonopore located on the anterior aspect of the 8th abdominal segment mid-ventrally, in the form of a dot-like cleft (Fig. 1). The determination of the segment on which the genital opening is located may be facilitated by pinpointing it from the wing pads which extend to the posterior margin of the fourth abdominal segments as shown in the figure.

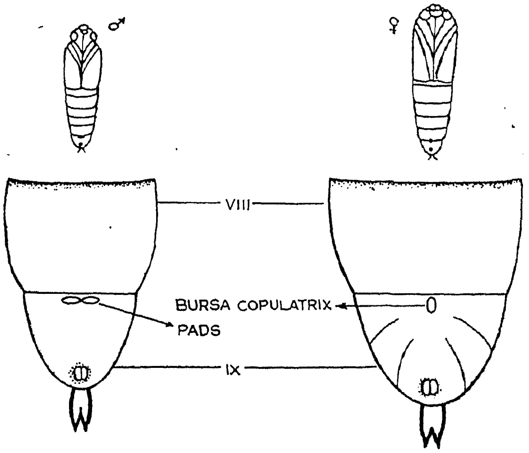

FIG. 1. Sexual dimorphsim in the pupa of *Heliothis armigera* Hbn.

It is evident from Table I that there was a significant difference in the weight, length and width of the pupae of both the sexes. The male pupae were much smaller than the female. Significant correlation was found to exist among all the three characters in either sex. The strength of the association was maximum between the weight and the length, the correlation coefficient (r) being 0·83 and 0·48 in female and male respectively. Significant correlation was also found between width and weight and width and length of pupae of the sexes. The development period of the pupae also varied significantly in the sexes, It lasted 11-13 days with an average of 12·0 days in the male and 10–11 days with a mean of 10·1 days in the female. It was also noticed that the male pupae showed constant twisting and turning movements whereas females were inactive and displayed only occasional movements.

TABLE I

Sex differences in size and weight of pupae of H. armigera (*Mean of* 20 *observations*)

Sex	Weight (mg)	Length (mm)	Width (mm)	Duration (days)
Male	222·58	16·36	4·89	11·95
Female	283·22	18·11	5·43	10·10
Standard error (S.E.)	23·87	1·36	0·50	0·17

The above characters provided an accurate method of separation of the two sexes. The adults also showed dimorphism by way of their external colour variations, the male being paler than the female which is brown in colour.

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---

1. Boles, H. P. and Mozke, F. O., In *Insect Colonization and Mass Production*, (Ed. C. N. Smith), Academic Press, New York, 1966, pp. 265.
2. Narayanan, K. and Jayaraj, S., *Symposium on Oriental Entomology*, Calcutta, Abstract, 1973, p. 40.
3. Sithananthan, S. and Subramaniam, T. R., *Madras agric. J.*, 1975, **62**, 62.

THE GRASS SEED INFESTING THRIPS *CHIROTHRIPS MEXICANUS* CRAWFORD ON *PENNISETUM TYPHOIDEUM* AND ITS PRINCIPAL ALTERNATE HOST *CHLORIS BARBATA*

MANY Gramineae and Cyperaceae in cultivated fields provide a perennial source of species of *Chirothrips*, *Anaphothrips*, *Bregmatothrips*, *Caliothrips* and *Haplothrips* to mention some of the major genera, are often of considerable importance in view of their build up in many crops at different stages of their growth. Species of *Chirothrips* have been known to feed on the ovarian tissues of many grass species destroying a good proportions of the seeds. Of such well-known species of *Chirothrips* are *C. mexicanus*, *C. falsus* Priesner producing a considerable decrease in the yield of Bermuda grass seed (Roney, 1949)[1] and Rhodes grass seed (Riherd 1954)[2] in the United States of America, of Timothy grass seed by *C. manicatus* Haliday and *C. hamatus* Trybom in Sweden (Johannson, 1946)[3] of meadow fox tail grass by *C. hamatus* in Finland (Hukkinen 1936)[4] and of cocksfoot seeds by *C. pallidicornis* Priesner in New Zealand (Doull, 1956[5]; Lewis, 1973[6]).

In so far as has been known the species *C. maximi* Ananthakrishnan, *C. ramakrishnai* Ananthakrishnan, *C. mexicanus* and *C. meridionalls* Bagnall form the major grass infesting thrips in India. While reporting for the first time the damage potential of the polyphagous cosmopolitan species *C. mexicanus* to young, growing ears of *Pennisetum typhoides*, observations presented herein also attempt at a correlation between their abundance in *Pennisetum* with that of its major alternative host *Chloris barbata*, abundant in *Pennisetum* fields. The incidence of this species in *Chloris barbata* is of the range of 25–30 individuals/branch of the four-branched spike, each with 44–50 spikelets, presenting an unusual preponderance of males to the extent of resulting in a sex ratio of 2:1 to 5:1 (males: females), appears to be of considerable interest suggesting an arrhenotokous parthenogenetic mode of reproduction occurring side by side with normal sexual reproduction.

Regular collections of *Chloris barbata* inflorescence as well as on the young growing, maturing and scenescent earheads of *Pennisetum*, provided evidence of the abudnance of *Chirothrips mexicanus* with population counts ranging from 30–48 in young earheads and decreasing to 22–32 in ripening earheads/25 beats, with scarcely an individual in the seenscent earheads the corresponding population counts in *Chloris barbata* range from 10–197 adults/25 beats (Fig. 1)

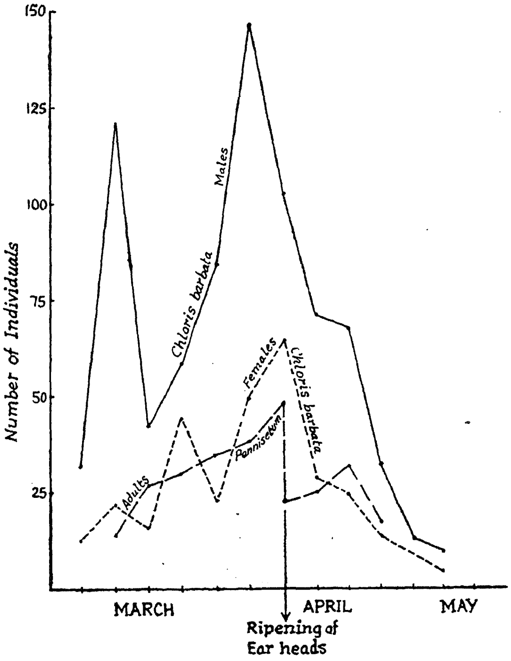

FIG. 1. Trends of infestation of *Chirothrips mexicanus* on *Pennisetum typhoides* and *Chloris barbata*

Only one egg is laid per floret, the larva feeding on the growing ovarian tissue, preventing seed formation. It was earlier reported in *C. pallidicornis* (Doull, 1956)[5] that, when the populations of *Chirothrips* reach 20/inflorescence, 30% cocksfoot seeds got destroyed. Examination of the inflorescence of *Chloris barbata* reveal all stages of development from egg to pupa with one individual per flower. Of 176–200 florets of *Chloris barbata* examined on a complete branched spike, 57–75 individuals of *C. mexicanus* occurred in various stages of development, there being only one individual per floret. The overall seed damage in *Pennisetum* was of the order of 5–10% of the cases, while in *Chloris barbata* the very heavy incidence indicated a total damage of 60% of the seeds. In view the ability *C. mexicanus* to maintain a steady population in this weed host *Chloris barbata*, the possibility of a very heavy build up in *Pennisetum* ears cannot be overlooked. Further work in this direction is in progress.

This work was carried out during the tenure of ICAR gr .nt for which thanks are due.

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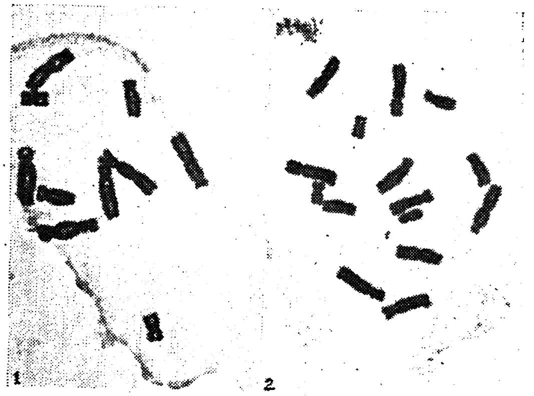
---

1. Roney, J. N., *J. Econ. Ent.*, 1949, **42**, 555.
2. Riherd, P. T., *Ibid.*, 1954, **47**, 709.
3. Johannson, E., *Meddn. St. Vaxtsa Amst.*, 1946, **46**, **1**.
4. Hukkinen, Y., *Valt. Maatalouskoet, Julk*, 1936, **81**, 1.
5. Doull, K. M., *N.Z. Jl. Sci. Technol.* (*A*), 1956, **38**, 56.
6. Lewis, T., *Thrips, Their Biology, Ecology and Economic Importance*. Academic Press, London, 1973.

CHROMOSOMES OF DIPLOID AND TRIPLOID *PUSCHKINIA LIBANOTICA* L.

Puschkinia libanotica L. (Fam. Liliaceae) has got five pairs of easily distinguishable chromosomes (Fig. 1). So far no heterochromatin has been detected in these chromosomes[7]. A few triploids were identified for the first time in *Puschkinia* (Fig. 2). Triploids appeared in the population with extremely low frequency. Of 2000 bulbs scored only 9 triploids without B chromosomes were isolated. Their chromosome length, chromosome volume, chromosome mass and DNA content were estimated and compared with diploids (Table I).



FIGS. 1–2. Eig. 1. Chromosomes of *Puschkinia*; $2n=10$ (× *ca.* 2,300). Fig. 2. Triploid in *Puschkinia*; $2n = 15$ (× *ca.* 2,000).

TABLE I

Chromosome length and volume, chromosome mass and DNA content

	Total chromosome length (micron)	Total chromosome[1] volume (cubic micron)	Chromosome mass[2] (× 10^{-11} gm)	DNA content[3] (arb. unit)
Diploid	71·59	191·64	19·37	18·03
Triploid	110·79	290·56	29·73	27 24

1. Chromosome volume measured at metaphase from 40 well spread cells (Figs. 1, 2) considering chromatids as cylindrical in form.
2. Chromosome mass = Total dry mass — nucleolar dry mass; estimates were made by interference microscopy (Davies[2]) in 40 2C nuclei isolated from root tips following the method of McLeish[4].
3. DNA content estimated by Feulgen photometry (McLeish and Sunderland[5] using Barr and Stroud Integrating Microdensitometer. 40 2C nuclei were estimated.

These characters in triploids showed a proportionate increase at a constant rate of 1·5 times as compared to diploids. This indicated that there was no change in chromosome length, chromosome volume, its mass and DNA content consequent to polyploidy. It was therefore, concluded that no chromosome reorganisation took place during the process of polyploidisation. On the other hand, several genera of Liliaceous family such as *Trillium*, *Fritillaria* and *Paris* showed diminution of chromosome size[1], where the chromosomes are characterised by significant amount of heterochromatin. La Cour's[3] observations in *Trillium tschonoskii* are also significant, where reduction of heterochromatic segments was noted in tetraploids. In the light of present observations it can be suggested that the effect of polyploidy is different in chromsomes with heterochromatin from those without.

Sharma[6] also observed no variation in chromosome size in tetraploids of *Vicia sativa* where no significant amount of heterochromatin was observed in the chromosomes of diploids. *V. faba* on the other hand having prominent heterochromatic segments in diploids showed significant difference in chromosome size in polyploids. From the present comparison, it therefore follows, that species where diploids do not have any significant amount of heterochromatin may not show any difference in chromosome size with increased level of ploidy.

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---

1. Darlington, C. D. and La Cour, L. F., *J. Genet.*, 1940, **40**, 185.
2. Davies, H. G., *General Cytochemical Methods*, Vol. I, Academic Press, Inc, 1958.
3. La Cour, L. F., *Heredity*, 1951, **5**, 37.
4. McLeish, J., *Proc. Roy. Soc. B.*, 1963, **158**, 261
5. — and Sunderland, N., *Exptl. Cell Res.*, 1961, **24**, 627.
6. Sharma, A. K., *Chromosomes Today*, 1972, **3**, 248
7. Vosa, C. G, *Ibid.*, 1969, **2**, 189.

KARYOTYPE IN THREE CULTIVATED VARIETIES OF *GLYCINE MAX* (L.) MERR.

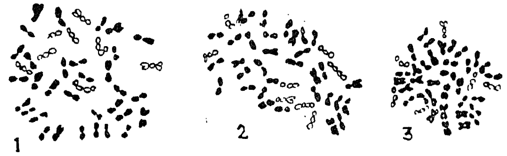
Glycine max (L.) Merr. commonly known as soyabean, is cytologically not adequately explored[3,4,5]. A thorough analysis of karyotype was made only recently in the cultivated variety UPI in *G. max*[1]. In this investigation a comparative study has been made on the detailed cytology of UPI, Improved Pelican and Mammoth. Somatic chromosomes were studied from young root-tips after pre-treatment in saturated paradichlorobenzene solution at 15° C for 2·5 hours and fixation in acetic-alcohol (1 : 2) for 30 minutes followed by hydrolysis in 2% aceto-orcein NHCl (9:1) soluton and finally squashing in 1% aceto-orcein solution. Somatic complement in this species has been found to consist of forty chromosomes (Figs. 1, 2, 3, 7). Secondary constrictions were observed in three to four pairs of chromosomes. The longest pair was provided with supernumerary constrictions. In all the varieties the karyotype was graded and the following morphological types have been observed (Figs. 4, 5, 6).

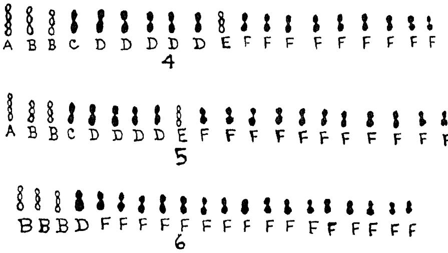
Type A—Represented by comparatively long chromosomes, each with three constrctions, one primary and the other two secondary. Of these, one was nearly median in position and the other two located in submedian positions at the two ends.

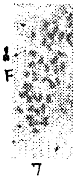
Type B—Represented by medium-sized chromosome each with two contrictions, primary and secondary. One of the constrictions was submedian in position and the other was nearly median to submedian to the longer arm.

Type C—Nearly long chromosomes each with median to nearly median primary constrictions.

Type D—Medium sized chromosomes of slightly ranging length each with submedian primary constrictions.




A B B C D D D D D E F F F F F F F F F F
4
A B B C D D D D E F F F F F F F F F F F
5
B B B D F F F F F F F F F F F F F F F F
6



FIGS. 1–3. Somatic metaphase in varieties UPI, IP and Mammoth respectively (2 n = 40), × 2,100.

FIGS. 4–6. Idiogram in UPI, IP and Mammoth respectively.

FIG. 7. Photomicrograph (2n = 40) in UPI.

Type E—Medium-sized chromosomes each with a primary and a secondary constriction. One of them was submedian in position and the other was nearly median to the longer arm.

Type F—Medium sized to short chromosomes of varying sizes, each with nearly median to submedian primary constrictions.

In the variety UPI, the chromosomes were short to medium-sized ranging from 1·5–3·2 μ, four pairs of them had secondary constrictions and the longest pair having supernumerary constrictions (Figs. 1, 4). All the six morphological types were present in this variety. Two pairs of chromosomes represented type B, one pair of them was comparatively long. Type D was represented by five pairs of chromosomes, two pairs had nearly submedian and three pairs had nearly median primary constrictions. The chromosomes in the variety Improved Pelican were mostly similar to those in the variety UPI having the size range between 1·4–2·4 μ. One pair of chromosome resembled type A but one of the constrictions was located at the distal end. The somatic chromosome complement in the variety Mammoth was quite different from that in the other two varieties; chromosome size in this variety varied from 1·3–2·3 μ. Types A, C and E are absent but B was represented by three pairs of chromosomes, of which one pair was provided with a very short secondary constriction at the distal end of the longer arm. Total amount of chromatin content per haploid complement was 41·4 μ in the variety UPI, 37·4 μ, in Improved Pelican and 36·1 μ in Mammoth.

Karyotype of *G. max* was not thoroughly analysed[4,5]. Detailed karyotype was analysed in *G. max* variety UPI following the technique described in ref. 1. In soyabean, however, a better karyotype was obtained following pretreatment in saturated paradichlorobenzene at 15° C for 1·5–2 hours and fixing in 3: 1 for 48 hours at 37° C before hydrolysis and subsequent staining in leuco-basic fuchsin. Besides, the root-tips were incubated in pectinase for 1·5 hours at 30° C[2].

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1. Biswas, A. K. and Bhattacharyya, N. K., *Cytologia*, 1972, **37**, 605.
2. Palmer, R. G. and Hollys Heer, *Crop Sci.*, 1973, **13**, 389.
3. Pritchard, A. J. and Gould, K. F., *Div. Trop. Pastures Tech. Pap.* 2 (C.S.I.R.O., Australia), 1964, p. 18.
4. Sen, N. K. and Vidyabhusan, R. V., *Euphytica*, 1960, **9**, 317.
5. Veatch, C., *Botanical Gazette*, 1934, 96, 189.

PHOTO-INDUCED CONIDIATION IN *ALTERNARIA MACROSPORA* ZIMM. AND *COLLETOTRICHUM GOMPHRENAE* RAO AND SALAM

BOTH quantum and quality of light influence sporogenesis in phytopathogenic fungi[1-5]. But the precise quality of light on sporogenesis has remained elusive till recently. Notable is the near ultraviolet (NUV) light which appears to be effective in inducing sporulation of a few fungi[6-8].

In this note we report on the quality of light influencing sporulation of two fungi. *Alternaria macrospora* Zimm. isolated from *Gossypium hirsutum* and *Colletotrichum gomphrenae* Rao and Salam from *Gomphrena decumbens* sporulated profusely when exposed to 'Black light' fluorescent lamp (320–420 nm) and 'cool white' day light fluorescent lamp (300–700 nm) respectively. *Alternaria macrospora* produced maximum number of spores when exposed to near ultraviolet light, but cultures incubated in daylight fluorescent lamp produced less number of spores (Table I). Several species of *Alternaria* sporulate profusely under near ultraviolet light followed by a period of darkness[9,10].

Of the various media tried, *viz.*, potato dextrose agar, water agar, oatmeal agar, cotton leaf extract agar, Czapek's agar with and without yeast extract (0·1%) and Richards' agar, we found that photo-induced sporulation in *A. macrospora* was more pronounced when grown on modified Richards' agar medium. Cultures incubated in total darkness did not sporulate in all media. Furthermore, lowering the sucrose concentration from 5·0–0·5% in Richards' agar induced the maximum number of spores.

TABLE I

Effect of 'Black Light' and 'Cool white' daylight fluorescent lamps on sporulation of A. macrospora *in culture**

Treatment	Spore No. /ml in thousands**
'Black light' lamp	6200
'Cool white' daylight lamp	20
Total darkness	nil

* 12 h light/12 h dark cycle was given and grown on Richards' agar.

Spore counts taken on 10th day after inoculation.

**Each result is an average from three replicates.

Colletotrichum gomphrenae grown on potato dextrose agar sporulated profusely when exposed to visible white light. But, near ultraviolet light from 'Black Light' was not as effective as light from visible white light. Leach[6] concluded that most fungi do not respond to near ultraviolet light in sporulation. Recent report on the effect of visible white light on enhanced spore production by *Trichometasphaeria turcica*[11]-agrees with Leach's statement. The response elicited by *C. gomphrenae* also clearly confirms to Leach's[6] conclusion.,

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1. Leach, C. M., *Can. J. Bot.*, 1961, **39**, 705.
2. Aragaki, M., *Phytopathology*, 1962, **52**, 122
3. Honda, Y., *Bull. Inst. agric. Res.*, Tohoku Univ 1969, **21**, 63.
4. Kumagai, T. and Oda, Y., *Devl. Growth and Diff* 1969, **11**, 130.
5. Bhama, K. S. and Swamy, R. N., *Kavaka*, 197 **1**, 19.
6. Leach, C. M., *Methods in Microbiology*, Vol. I ed. by C. Booth, Academic Press, New Yo and London, 1971, p. 609.
7. Calpouzos, L. and Stallknecht, G. F., *Phyt pathology*, 1967, **57**, 679.
8. Tan, K. K. and Epton. H. A. S., *Trans. Br mycol. Soc.*, 1973, **61**, 147.
9. Leach, C. M., *Can. J. Bot.*, 1967, **45**, 1999.
10. Lukens, W. J., *Am. J. Bot.*, 1963, **50**, 720.
11. Benedict, G. G., *Can. J. Bot.*, 1976, **54**, 552.

CALLUS INDUCTION AND BUD FORMATION IN *FURCRAEA GIGANTEA* VENT., AMARYLLIDACEAE

ESTABLISHMENT of plantlets *in vitro* in monocotyledons is restricted to members of a few families like Gramineae, Orchidaceae, Liliaceae and Iridaceae, which have been ably summarised recently[1-3]. The comparative study of the totipotency in tissue explants and callus of some members of Liliaceae, Iridaceae and Amaryllidaceae show that the morphogenetic response of the latter was not encouraging[3].

The present investigation on *Furcraea gigantea* Vent., a fiber-yielding plant of Amaryllidaceae is to report the regenerating capability and totipotency of the cells for callus induction and bud differentiation *in vitro*.

Several bulbils having the potentialities to develop into independent plants were produced on the huge branched inflorescence axis. Each bulbil had a basal swollen part gradually tapering to the apex, ensheathed by three or four leaves. The basal part of the bulbil after proper sterilization was cut into four longitudinal sectors under aseptic conditions. The explants were grown on the Murashige and Skoog medium[4] supplemented with kinetin (1·2 mg/1), IAA (4 mg/1) and, 3% sucrose as carbohydrate source. The medium was jelled with 1% Bacto Difco Agar. Prior to autoclaving, the pH was adjusted to 5·8. Cultures were maintained at 28 ± 2°C and with an intensity of 1200 lux light for 8 hours in 24 hours period.

In 13 weeks, the explants regenerated into plantlets without callus formation. These regenerated plantlets were morphologically different from the bulbils, in having a thin basal part ensheathed by many narrow leaves. The basal part of the leaves from the regenerants were excised and transferred to Norstog and Rhamstine medium 59 (2)[5] supplemented with coconut milk (150 ml/1) and 2, 4 -D (0·5 mg/1) and with the omission of adenine sulphate and kinetin. It was jelled with 0·8% Bacto Difco Agar. Physical conditions were maintained as before.

The pale-yellow callus, developed after two weeks of inoculation, became quite profuse at the end of the 5th week and the condensed buds were differentiated. In 13 weeks, the buds developed further and produced many long and narrow leaves. However, rhizogenesis could not be observed even after a long continuous culture.

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1. Hussey, G., *J. ex. Bot.*, 1975, 26, 253.
2. Vasil, I. K. and Nitsch., C., *Z. Pflanzenphysiol.*, 1975, 76, 191.
3. Kato, Y., *Ibid.*, 1975, 75, 211.
4. Murashige, T. and Skoog., F., *Physiol. Plant.*, 1962, **15**, 473.
5. Norstog, K. and Rhamstine, E., *Phytomorphology*, 1967, **17**, 374.

POST-INFECTION CHANGES IN VITAMIN C CONTENTS OF BANANA FRUITS

THE deficiency of L-ascorbic acid is known to produce scurvy in human beings. It is required in several metabolic pathways and its biosynthesis in plants has been extensively reviewed by Mapson[5] and Isherwood and Mapson[3].

Helminthosporium speciferum Bain (Nicot) causes considerable rotting of banana fruits in local market and storage. Ascorbic acid contents in rotting 'alpan' bananas were determined by inoculating *H. speciferum.* Six replicates were used for each set of experiment. Inoculated fruits were incubated at 25° ± 1°C for 15 days and healthy and diseased tissues adjacent to the inoculated region were analysed at regular intervals of 5 days. Quantitative estimation in ascorbic acid content was made by Systronics colorimeter. Modified method suggested by Roe and Kuether[6] was employed and the results are presented in Table I.

TABLE I

Showing ascorbic acid (mg/100 gm. of fruit pulp) of controlled and infected fruits of banana

	Ascorbic acid				% of loss in ascorbic acid in 15 days of incubation
	Days of incubation				
	0	5	10	15	
Control	45·6	40·6	28·5	21·4	53·3%
Inoculated with *H. speciferum*	..	30·6	22·26	13·3	70·8%

Table I indicates a gradual loss in vitamin C in both the healthy and infected fruits. However, the fall was comparatively slower in control fruits. The percentage loss in fruits infected with *H. speciferum* was 70·8% while corresponding loss in healthy fruits was 53·3% only. Many investigators[2,4,7] working with common Indian fruits and vegetables have made similar observations in fruits under pathogenesis. Ascorbic acid functions as one of the biologeical oxidation-reduction substances. It is easily oxidized to dehydro-L-ascorbic acid by the enzyme ascorbic acid oxidase or by certain other oxidative enzymes.

It seems, therefore, probable that the decline in the ascorbic acid is due to the production of ascorbic acid-degenerating enzymes either by the fungus itself or by the host-parasite interaction as postulated by Ghosh *et al.*[1].

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Bhagalpur, *November* 15, 1976.

---

1. Ghosh, A. K., Tandon, R. N., Bhargava, S. N. and Srivastava, M. P., *Naturwissenschaften*, 1965, **52**, 478.
2. —, Bhargava, S. N. and Tandon, R. N., *Indian Phytopathol.*, 1966, **21**, 262.
3. Isherwood, F. A. and Mapson, L. W., *Ann. Rev. Plant Physiol.*, 1962, **13**, 329.
4. Kapoor, I. J. and Tandon, R. N., *Curr. Sci.*, 1969, **39**, 397.
5. Mapson, L. W., *Ann. Rev. Plant Physiol.*, 1958, **9**, 119.
6. Roe, J. H. and Kuether, C. H., *J. Biol. Chem.*, 1943, **147**, 399.
7. Tandon, M. P. and Jamaluddin, *Curr. Sci.*, 1973, **42**, 400.

RADIATION-GENETIC STUDIES IN GARDEN PEA: TWO EARLY FLOWERING AND RIPENING INDUCED MUTANTS

Isolation of early flowering and ripening mutants is one of the most important goals in pea breeding. A few early mutants in *Pisum sativum*, the garden pea, have been reported. The earliness in these mutants is due to the formation of the first inflorescence at the nodes which are formed early in the development of these plants. The flowers in these mutants are produced in the axil of 4th–6th instead of 11th–13th leaf. In comparison to their respective initial lines, most of these mutants flower 10–14 days earlier, but their seed production gets significantly reduced[1].

In a radiation-genetic experiment, in which the seed of Bonneville variety of pea were utilized[5], early plants segregated in two M2 lines and they bred true in M3 and M4 generations. Crossing with the initial line indicated a monogenic recessive control of the earliness in these. Intercrossing between these two early mutants indicated that the two genes inducing earliness were non-allelic, as the F1 was not early unlike the parents. In 1975, 40 plants of each of these two mutants, the initial line, the control, the local line and Prof. Gottschalk's early mutant 46C were grown in a randomised block in 5 replications (each replicate comprised of 40 plants) at two locations, Kurukshetra (N. India) and Shillong (N.E. India); the plant to plant and row to row distance in each case was 10 and 30 cm, respectively. Critical difference for each trait was computed following the usual analysis of variance technique.

Yield and other agronomic characters of these mutants are given in Table I, a perusal of which reveals that EM1 and EM2 differ significantly in morphological traits from 46C. Unlike 46C, they also exhibit a perfectly normal floral structure and physiological earliness. At Kurukshetra, EM1 was significantly shorter and more productive than its initial line and 46C (Table I). However, at both the locations, 46C and EM1 were earlier in flowering and ripening than the initial and the local line. All the genotypes (except EM1) became dwarf, flowered and ripened later, and, yielded fewer seeds at Shillong than at Kurukshetra (Table I). This differential behaviour of the genotypes at these two locations could be a expression of "place or location effect". At Shillong both the early mutants exhibited a significantly highe seed production than the initial and local line; highe seed production being in EM1. In fact, EM1 is th best productive early genotype at both the locati and represents one of the potential genotypes to be us for pea breeding in these two regions. Unfortunatel due to late sowing, the pea genotypes were attacked *Erysiphae polygoni* DC. and *Perenosporapisi* Syd., the fruiting stage at Shillong. This also reduc their total grain production.

While Snoad and Arthur[7] found that early floweri from the accumulation of dominant alleles, Ro lands[6] and Snoad and Arthur[7] reported the resul be due to an accumulation of recessive alleles. W *et al.*[9] regarded that the early flowering in garden was due to an accumulation of recessive alleles, the genetic system was mainly additive in eff However, their regression of Wr on Vr after Hayn indicated the existence of full dominance. The do nance was lower in F2 than in F1. Therefore, chances of recognising dominance were higher in rather than in F2. Analysing the basic pea mat used, it appears that while in the diallel crosse Rowlands[6], Watts *et al.*[9], and Snoad and Arth only European and North American cultivars utilised, out of 6 parents used by Snoad and Art 4 were quite unrelated to European cultivars these, therefore, exhibited differences in the sy of genetic control of some traints. This is obv

TABLE I

*Performance of early mutants, initial line and the local lines of garden pea at two locations**

Location	Genotype	Shoot height (cm)	Total nodes per plant	Mean inter-node length (cm)	Days to first flowering	Days to ripen	Pods per plant	Seeds per pod	Seeds per plant	1,000 grain weight (g)
Kurukshetra	Initial line	94·1	21·2	6·2	68	138	12·8	4·5	58·2	97·5
	Early mutant I (EM1)	68·3	19·4	3·5	52	121	11·4	3·8	44·3	160·0
	46 C	125·4	25·1	4·9	55	127	14·0	1·8	25·3	165
	Local line	133·4	21·2	3·9	66	137	15·2	2·8	44·0	162
	Critical difference value at 5% P level	8·3	2·7	2·7	5·3	8	2·1	0·3	4	5·7
Shillong	Initial line	69·8	14·9	4·5	117	170	5·01	2·8	14·0	113·4
	46 C	70·4	11·1	6·3	83	134	3·60	2·9	10	175·8
	Early mutant I (EM1)	65·3	14·3	4·5	77	128	6·70	5·2	35	172·3
	Early mutant II (EM2)	64·4	14·1	4·5	86	133	4·92	4·2	21	230·2
	Local line	120·9	17·7	6·8	112	173	5·90	3·4	20·0	215
	Critical difference value at 5% P level	9·7	2·6	1·3	3·7	11·4	2·7	0·4	3·4	5

* (Mean value of 200 plants; 40 plants each in five replications.)

from the fact that even with European and North American cultivars there have been suggestions of different genetic systems governing flowering time. For instance Cousin[2], from the analysis of all-cultivar diallel crosses, has indicated the presence of second polygenic system in which earliness of flowering is due to an accumulation of dominant alleles. Watts *et al.*[9] demonstrated that the late flowering in the variety "*Jade*" was the cause of gene interactions tending to increase earliness of flowering in combination with late flowering cultivars and delayed the flowering in combination with earlier flowering types. In the present material, mutation of specific dominant genes to their recessive forms resulted in earliness, the two early genes isolated are non-allelic indicating thereby a multiple allelic control of earliness in the garden pea.

Earliness and lateness, and dominance and recessiveness, being relative terms, can be compared when the results from two different sets of diallel crosses with only one common parent are available. In the control of flowering time of pea, major and subtle differences seem to exist. The system appears to be under more than one genetic control. Therefore, for breeding purposes, it is vital to screen extensively, both, the primitive and the cultivated group of peas, with a hope of determining different genetic systems controlling other traits of breeding. The differences already revealed even in one the control of flowering time, indicate the fragme nature of the evidence which is obtainable such small sets of diallel crosses. These also show unwise it would be to extrapolate beyond the par used, in any one experiment since only a porti the whole range of genetic variability is sampled each occasion. Finally, the results which are ob at different times and in different places are inevit influenced by genotype-environment interactions are operative all through. Therefore, the differe behaviour of mutant genes in garden pea at N. and N.E. India is a pointer towards mutant environment interaction and needs to be further lysed. However, preliminary studies on the logical investigations in *Pisum* mutants by Gott and Kaul[3] have revealed facts of interest and im tance for pea breeders.

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---

1. Blixt, S. and Gottschalk, W., *Agri. Hortique Genet.*, 1975, **33**, 33.
2. Cousin, R., In *Proceedings of the Eucarpia Conference on Peas*, INRA, Versailles. June 1972.
3. Gottschalk, W. and Kaul, M. L. H., *Z. Pflanzenzuchtg*, 1975, **75**, 182.
4. Hayman, B. I., *Genetics*, 1953, **39**, 789.
5. Kaul, M. L. H. and Matta, N. K., *Genetika*, 1976, **8** (in press).
6. Rowlands, D. G., *Genetica*, 1964, **35**, 75.
7. Snoad, B. and Arthur, A.E., *Euphytica*, 197 , **22**, 327.
8. — and —, *Ibid.*, 1973, p. 510.
9. Watts, L. E., Stevenson, E. and Crampton, M. J., *Ibid.*, 1970, **19**, 405.

INHIBITION OF RUSTS OF IRIS AND WHEAT BY TWO PHYTOPATHOGENIC PSEUDOMONADS

Pseudomonas marginata (McCulloch) Stapp has been reported to initiate infection on iris leaves through rust pustules[1]. As the rust pustules were acting as principal avenues for the entry of the bacterium in host leaves, it was considered intresting to know the influence of the association on the rust fungus [*Puccinia iridis* (DC) Wallr.]. Germination tests were, therefore, conducted with uredospores collected from apparently normal and bacteria infested rust pustules. A remarkable reduction of germination of uredospores was detected in the latter case. A similar phenomenon was also observed to occur when non-contaminated uredospores of iris rust were allowed to germinats in a drop of aqueous suspension of 48 hr old culture of *P. marginata*. It was, thus, indicated that the contamination of rust uredospores with *P. marginatae* inhibited the germination of iris rust uredospores, in nature.

To confirm the inhibition of germination by *P. marginata*, uredospores of rusts of iris and wheat, (*P. graminis* f. sp. *tritici* and *P. recondita*) were kept for termination on glass slides in drops of bacterial suspension in a dilution series. The test in drops of water and an aqueous suspension of *P. sesami* served as checks. Inhibition of germination occurred with *P. marginata* only. In the drops with higher bacterial concentrations, the inhibition was complete whereas in the cases of lower concentrations, the uredospores did germinate but the growth of germ-tubes was remarkably retarded. For further microscopic examination, the drops on slides were air dried and stained with crystal violet bacteria staining solution. In drops with *P. marginata*, a congregation of bacterial cells was observed on the surface of uredospores and germ tubes. Heavy congregation of bacterial cells seemed to effect a total inhibition of uredospore germination. In the cases where germination of spores had occurred, the bacterial cells collected in large numbers around the tips of germ tubes resulting either in a growth retarding effect on germ-tubes or bursting of their tips. In the case of *P. sesami*, congregation of bacterial cells occurred in the vicinity of tips of germ-tubes but without any obvious influence on these structures.

The inoculation of healthy leaves of iris and wheat with *P. marginata* contaminated uredospores of respective rusts, failed to produce any rust infection. A normal rust infection, however, developed on the host leaves inoculated with *P. sesami* contaminated rust uredospores. It was further observed that when a dilution series of *P. marginata* was employed to contaminate the rust uredospores, the development of rust infection on both the hosts showed a negative correlation with the concentration of bacterial cells in the dilutions. *P. marginata* was, thus, observed to inhibit the iris and wheat rusts identically.

With a view to detecting the rust-inhibiting phenomenon with other bacterial phytopathogens, germination tests were carried out with uredospores of stem rust of wheat in drops of aqueous bacterial suspension of 8 cultures of *Pseudomonas*, 6 cultures of *Xanthomonas* and one culture each of *Agrobacterium tumefaciens* and *Erwinia carotovora*. Germination of uredospores in aqueous suspensions of *Eschrichia coli* and *P. marginata*, and water alone served as checks. The culture of *P. cichorii* (Swingle) Stapp, inciting a zonate spot on cabbage, was identified to act as strong inhibitor of germination of uredospores of stem rust of wheat.

The present investigation records *P. marginata* and *P. cichorii* as antagonistic to rusts of iris and wheat. Adequate information is needed on the mechanism of inhibition and the scope of utilizing these organisms for the control of plant rusts.

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1. Durgapal, J. C. and Trivedi, B. M., *Curr. Sci.*, 1976, **45**, 562.

GENOTYPIC CONTROL OF CHIASMA TERMINALIZATION IN RADISH

GENOTYPIC control of chromosome characters, particularly chiasma frequency, has been demonstrated in a number of plants, including radish[1-7]. Cytological study of chiasmata is more informative than the genetic analysis of meiotic recombination since it does not only specify the mean recombination frequency for the whole genome, but also its distribution pattern in the genome. Further, cytological study of mutants or inbred lines of an outbreeding population may provide an insight into the mechanism of chromosome pairing, one of the most important problems in cytogenetics.

In the present report an attempt has been made to study the mean number and the terminalization coefficient of chiasmata in three inbred lines of radish (*Raphanus sativus* L. var. *radicola* Pers.), namely, LS–377/24 (I_{11}), LS–337/25 (I_{11}) and LS–43/51 (I_{15}), their F_1 hybrids and the original population in order to understand the genetics of chromosome pairing, particularly chiasma terminalization.

Materials and methods of investigation remain essentially the same as described elsewhere[6-7].

Inbred lines of radish differ significantly among themselves as well as from the original population in mean chiasma frequency at both diakinesis and metaphase I of the meiotic cell division. Mean chiasma frequency in inbred lines is significantly lower than the original population. In all the interlinear hybrids, on the other hand, there is a significant increase in mean chiasma frequency, touching almost the level of the original population, which may be regarded as a case of heterosis for this chromosome character.

As regards the coefficient of chiasma terminalization, its value is significantly higher at both the stages of meiosis in the inbred lines, particularly in LS–337/24 and LS–337/25, when compared to the original population. However, LS–43/51 behaves differently; at diakinesis it does differ from the population and the other two lines, but at metaphase I it differs only from the latter. Interestingly, interlinear hybrids behave like the original population in this character (Table I). In other words, it indicates that more chiasmata are terminalized at a particular stage of meiosis in the inbred lines than in the original population and interlinear hybrids. Thus, the chromosome pairing or synapsis is apparently relaxed in the inbred lines.

TABLE I

Mean chiasma frequency and coefficient of chiasma terminalization at diakinesis and metaphase I in the inbred lines of radish, their F_1 hybrids and the original population

Materials	Meiotic Stage	No. X'ta/cell Mean ± S.E.	No. terminalization X'ta/cell Mean ± S.E.	Koeff. X'ta terminalization. Mean ± S.E.
Population	Diak.	19·7±0·10	12·8±0·17	0·65±0·009
	M–I	17·3±0·07	15·4±0·13	0·89±0·008
LS–337/24 (I_{11})	Diak.	15·8±0·10	12·0±0·16	0·75±0·010*
	M–I	13·7±0·12	13·6±0·12	0·98±0·011*
LS–337/25 (I_{11})	Diak.	16·5±0·12	11·2±0·16	0·68±0·010
	M–I	14·3±0·13	13·8±0·11	0·98±0·007*
LS–43/51 (I_{15})	Diak.	16·8±0·10	11·2±0·14	0·67±0·008
	M–I	16·3±0·09	15·4±0·12	0·94±0·008*
LS–337/25 × LS–337/24	Diak.	17·9±0·08	12·3±0·14	0·68±0·008
	M–I	16·7±0·12	15·7±0·12	0·94±0·009*
LS–43/51 × LS–337/24	Diak	17·7±0·12	12·3±0·10	0·69±0·006*
	M–I	16·6±0·07	15·8±0·08	0·95±0·009*
LS–43/51 × LS–337/25	Diak.	17·7±0·09	12·0±0·11	0·68±0·006
	M–I	16·6±0·07	15·6±0·10	0·94±0·006*

* denotes significant differences from the original population.

In the present case, highly homozygous inbred lines of radish have been modelled for the study of chromosome pairing with particular reference to chiasma terminalization. Our study demonstrates a relaxed synapsis for the inbred lines, but this relaxation in chromosome pairing is withdrawn in their hybrids and the original population. Relaxation in chromosome pairing, which is so characteristic of the inbred lines, can be understood in the light of the model of chiasma control, proposed recently by Jones[8]. According to this model, each bivalent contains a very large number of potential sites for chiasma formation. These sites are evenly distributed along the entire length of the bivalent and have uniform but very low probability of chiamsa formation. It is quite probable that forced and prolonged inbreeding might affect these 'sites' in various ways; one of its consequence may be hurried terminalization of chiasmata in the inbred lines (homozygotes). However, this is normalized in the interlinear hybrids and the original population (heterozygotes). Thus, chiasma terminalization in radish is genetically conditioned and bears a definite relationship with homo- and heterozygosity of genes.

Our results also confirm an earlier report that mean chiasma frequency is significantly reduced in the inbred lines of radish in comparison to the interlinear hybrids and the original population[7]. This has been attributed to the heterozygosity of genes brought about by prolonged and forced inbreeding of an outbreeding and heterozygous population which radish constitutes.

I wish to express my sincere thanks to Prof. T. S. Fadeyeva and Dr. S. I. Narbut of the Chair of Genetics and Plant Breeding. Leningrad State University, Leningrad (U.S.S.R.), for suggestion and criticism.

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---

1. Rees, H., *Heredity*, 1955 *a*, **9**, 93.
2. —, *Proc. Roy. Soc., Ser. B*, 1955 *b*, **144**, 150.
3. Zecevic, L. M., *Zbor. radova Biol. Inst.*, 1961, **5**, 1.
4. Riley, R. and Law, C. N., *Adv. Genet.*, 1965, **13**, 57.
5. —, *Sci. Progress*, 1966, **54**, 193.
6. Dayal, N., *Caryologia*, 1975, **28**, 429.
7. —, *Cytologia*, 1956, **41** (In press).
Jones, G. H., *Heredity*, 1974, **32**, 375.

OCCURRENCE OF SEPTATE MACROSCLEREIDS IN THE BARK OF STEM OF *ANOGEISSUS LATIFOLIA* WALL

INTERNAL hairs (sclereids) in some species of Aroideae were reported to possess a few septa in the lumen[1]. Ananda Rao[2] reported such septa in the sclereids of *Scindapsus*. To our knowledge there is no other report on the occurrence of septate sclereids. In the bark of stem of *Anogeissus latifolia* short macrosclereids with septate lumen were observed very frequently (darts in Fig. 1 A–D). The wall of the sclereid shows

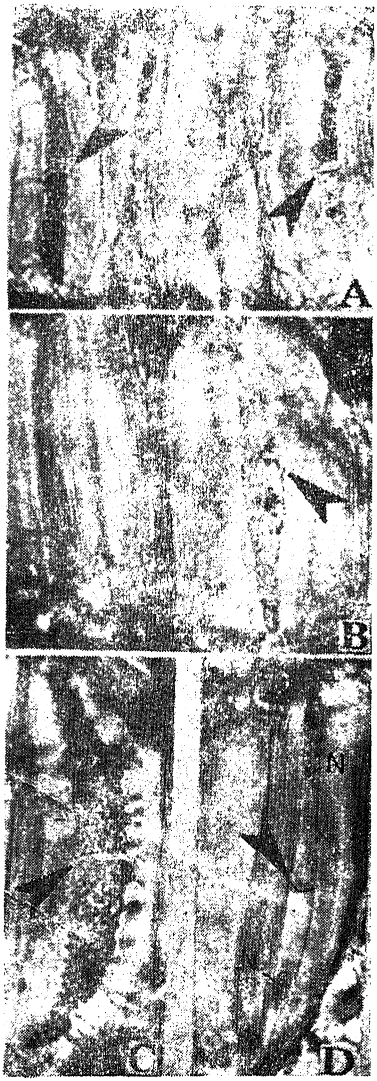

FIG. 1 A–D. Longisection of macrosclereids showing septa (at darts), nuclei (Fig. 1 D, *N*), and contents of the lumen. A, × 150; B, × 375; C, × 480; D, × 340.

striations of secondary wall and distinct pit canals (Fig. 1 C, D). The lumen is divided into two or three equal or unequal chambers by one or two thin septa. The septum extends only up to the innermost secondary wall layer. Earlier report shows the negative reaction to cellulose tests by such septa[2]. However, the thin septa are found to be PAS-positive in the present investigation (Fig. 1 A, D). The compartments possess one nucleus each (Fig. 1 D). The lumens of the sclereids may be broad (Fig. 1 C) or narrow (Fig. 1 D). Amorphous PAS—positive substance was also observed in the lumen of sclereids (Fig. 1 A, C). The earlier report of De Bary[1] and Ananda Rao[2] confine to long and branched sclereids resembling fibres. Unlike this, our observation, probably, is the first report of occurrence of septate macrosclereids.

This work was carried out under the UGC project on "Studies of sapwood and heartwood of forest trees of Gujarat".

Department of Botany, P. M. Eldo.
Sardar Patel University, J. D. Patel.
Vallabh Vidyanagar 388 120, J. J. Shah.
September 7, 1976.

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1. De Bary, A., *Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns*, Clarendon, 1884.
2. Ananda Rao, T., *Curr. Sci.*, 1954, **23**, 365.

CHOLESTEROL SYNTHESIS IN *COLLETOTRICHUM DEMATIUM* (PERS. EX. FR.) GROVE

Sterols are known to occur in dilute concentrations in a large number of fungi. Ergosterol, a fungi sterol is quite wide-spread and is known from diverse groups of fungi. Its amount is usually 1·0% or less of the total dry weight of the mycelium[1]. A comprehensive survey has been made by Pruess[2] and others[3]. The highest level (1·7%) so far reported is from *Pacilomyces variotii*. Cholesterol, which is a precursor of several metabolites and ergosterol have not been reported previously from the fungi while some other sterols (except ergosterol) have been tentatively identified. The present investigation reports cholesterol in four isolates of *Colletotrichum dematium*. The isolates were obtained from leaf spots of different medicinal plants. The isolates were grown in Asthana and Hawker's medium A and the mycelium was harvested after 10 days of incubation. Preliminary tests for cholesterol were done by the method suggested by Plummer[4] and quantitative estimations by colorimetry[5]. The results are presented in Table I.

TABLE I

Showing amount of cholesterol in the dried mycelial mats of different isolates of C. dematium

Isolates	Growth yield (in mg)	Total amount of Cholesterol in dried mycelium (mg.)	Percentage of cholesterol
I	89·4	1·143	1·28
II	82·0	1·180	1·44
III	92·2	0·885	0·96
IV	110·8	0·894	0·80

Interestingly, the highest percentage of cholesterol was an isolate (II) which had comparatively a poor growth rate, while the lowest percentage was in an isolate (IV) with maximum growth. This appears to suggest that higher cholesterol content retards growth in *C. dematium*. It may be mentioned that fluctuation in the percentage of sterols as seen here has previously been reported in different single spore strains of fungus[6, 7].

Further work is in progress.

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November 1, 1976

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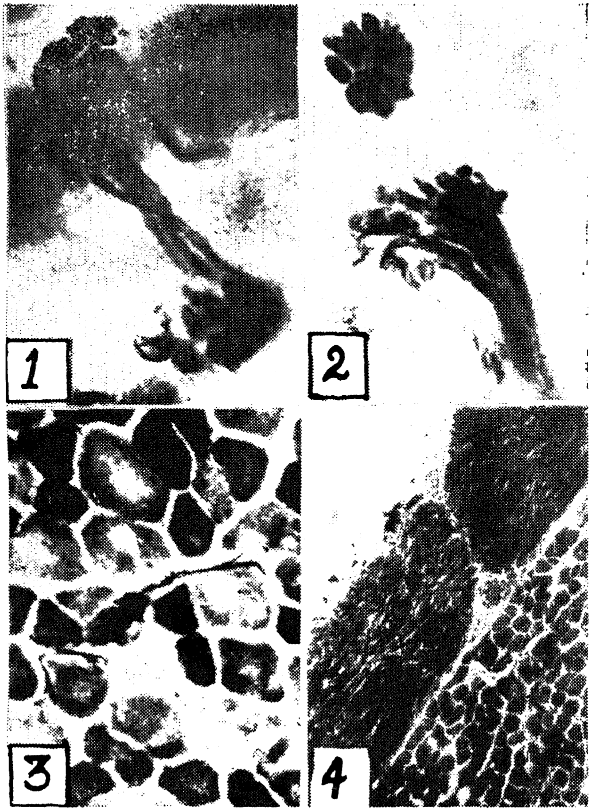
1 Cochrane, V. W., *Physiology of Fungi*, New York. John Willey and Sons, Inc., London, 1958, p. 48.
2. Pruess, L. M., Eichinger and Peterson, W. H., *Zentr. Bakteriol.*, 1934, **29**, 21.
3. —, Peterson, W. H., Steenbock, H. and Fred, E. B., *J. Biol. Chem.*, 1931, **90**, 369.
4. Plummer, T. P., *An Introduction to Practical Bio-Chemistry*, McGraw-Hill, 1971, p. 188.
5. Zarrow, M. X., Yochim, J. M. and McCarthy, J. L., *Experimental Endocrinology, A Source Book of Basic Techniques*, Academic Press, New York and London, 1964, p. 206.
6. Bernhauer, K. and Petzelt, G., *Biochem.* 2, 1935, **280**, 388.
7. Wenck, P. R., Peterson, W. H., and Greenne, H. C., *Zentr. Bakteriol. Parasitenk Abt.*, **11**, 1935, **92**, 324.

A HISTOCHEMICAL STUDY OF THE INNERVATION PATTERN IN MAMMALIAN SKELETAL MUSCLE BIOPSY

A STUDY of gross muscle fibre innervation is essential for the proper evaluation of skeletal muscle pathology[1,2] and also in the general study of motor units in skeletal muscles. Most of the histological/histochemical procedures employed for the study of muscle fibre innervation are elaborate and time-consuming.



FIGS. 1-4. Fig. 1. Longitudinal section of rat gastrocnemius showing the gross innervation pattern of red (darkly stained) and white (lightly stained) muscle fibres, × 200. Fig. 2. Same as in Fig. 1, showing innervation in a region containing only white muscle fibres, × 200. Fig. 3. Same as in Fig. 1. Note the distinct staining of the heterogeneous population of muscle fibres, and of the nerve fibres, × 50. Fig. 4. Same as in Fig. 1. Note the staining of the cross-section of the two branches of the sciatic nerve, and also of the muscle fibres, × 50.

More recently muscle innervation has been studied by combining the acetylcholinesterase and silver impregnation technique[3], and also by methylene ablue staining[4]. In the present investigation we have used a procedure that is rapid and serves the twin purpose of muscle fibre type identification and the visualization of their gross innervation.

16 μ thick fresh-frozen sections of the muscle pieces were fixed for 5 min in 1% cobalt chloride and calcium chloride made in 10% neutral buffered formalin. This fixative was found particularly suitable for the specific staining of nerves (fibres) in muscle sections, besides preserving intramuscular lipids to good effect. Following fixation, the sections were thoroughly washed for 1 min in running tap-water, and then for 1 more min in distilled water. Washed sections were stained for 10 min in a saturated alcoholic solution of Sudan Black B, rinsed in two ½ min changes of 30% alcohol, and then further washed in running tap-water for 2 min. Finally, the sections were mounted in glycerine-jelly. The overall processing time of the fresh frozen sections was never more than 20 min. For a better evaluation of the results of the present technique, both longitudinal and cross-muscle sections were used. By thie technique, however, the nerve fibres could not be precisely traced till the motor end-plate area. Otherwise, it was possible to observe the gross course of nerve fibres along the various muscle fibre types (Figs. 1-3). Whole nerve cross-sections could also be stained with good effect (Fig. 4).

It may be mentioned here that the present technique may prove useful in studying the fibre type innervation pattern of mixed or predominantly white muscles, and not of predominantly or exclusively red muscles (fibres) stain as dark with Sudan Black B as the nerves (fibres) do, and thus the overall staining contrast of the nerve fibres is insignificant. For the reason of its quickness and reproducibility, this technique may possibly be used for the biopsy studies of gross skeletal muscle innervation.

This study was supported in part by a research grant from the U.G.C., New Delhi, to one of us (C.L.T.).

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1. Adams, R. D., Denny-Brown, D. and Pearson, C. N., *Diseases of Muscle : A Study in Pathology*, 2nd Edn., Harper and Row Publishers, New York, 1965.
2. Woolf, A. L., In: *Disorders of Voluntary Muscle*, Edited by Walton, J. N., J. and A. Churchill Ltd., London, 1969.
3. Ip, M. C. and Vrbova, G., *Z. Zellforsch.*, 1973, **146**, 261.
4. Korneliussen, H. and Waerhaug, O., *Z. Anat. Entwickl. Gesch.*, 1973, **140**, 73.

SHORT SCIENTIFIC NOTES

A New Species of Genus *Septoria*, Fr. em. Sacc, from Kashmir

Acer caesium, Wall. ex. Brand, a large tree of the high altitude regions of Kashmir (between 7000–10,000 feet elevations) was observed to be severely attacked by *Septoria* spp. The infested material was collected in the Gurez Valley, Kashmir, at an altitude of 7200 feet during June, 1976.

The disease manifests as the dark-brown pin-heads arranged in clusters in necrotic zones on the leaves. The necrotic spots have faint brown margins and measure between 4·5–9·0 mm across. A few fruiting bodies may appear even outside the necrotic areas.

Examination of the specimen by Dr Punithalingum and Dr. M. B. Ellis, at the CMI, Kew, England, revealed that the pathogen did not agree with any known species of *Septoria*, and it could not, therefore, be assigned to any specific position. The species is hence described *de novo* and is designated as *Septoria gureza*, Mirakhur, Tahir and Dhar: spp. nov.

The fungus was isolated on PDA and Czapek's agar medium where it grew with profuse sporulation. Pycnidia of the natural specimens are conspicuous as dark-brown to black bodies, closely crowded, globose and visible to the naked eye. The conidia were long, slender, hyaline, and faintly septate. The diameter measurements of pycnidia: (160–) 200–240 (–250) μ, and the measurements of conidia: (50–) 55–60 (–65) × 1·5 – 2 μ.

The disease specimen has been deposited at the Commonwealth Mycological Institute, Kew, England, under reference No. IMI 205047.

The valuable help received from Dr. Punithalingum and Dr. M. B. Ellis of the Commonwealth Mycological Institute, Kew, England, is gratefully acknowledged The authors express their gratitude to Mr. A. G. Malik, Director of Agriculture, Jammu and Kashmir, for his keen interest.

Agricultural Research Station, R. K. Mirakhur.
Shalimar, Kashmir. H. M. Tahir.
October 8, 1976. A. K. Dhar.

Occurrence of *Fusarium chlamydosporum* on Virus Infected Papaya Plants

Pieces of stem, root and the rhizosphere soil from mosaic infected papaya plants (*Carica papaya* L.) were repeatedly plated on PDA to isolate the possible pathogen. After 6 days the plates were observed to contain a fungus. The fungus thus isolated was identified as *Fusarium chlamydosporum* Wollenweber and Reinking. The fungus was confirmed as pathogen only after satisfying the Koch's postulates. The culture has been deposited in the culture collection of Botany Department, Marathwada University, Aurangabad.

Resistance and susceptibility[1] to fungal pathogens by virus infected plants has been reported. The fungus *F. chlamydosporum* was reported[2] from Indian soils. However, this is the first reported occurrence of the fungus on virus infected papaya plants in India.

Authors thank Prof. K. B. Deshpande, for facilities and encouragement.

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1. Mahmood, K., Akram, M., Qamar, S., Naqvi, A. and Alam, M. M., *Indian Phytopath.*, 1974, 27 (4), 627.
2. Subramanian, C. V., *J. Madras Univ.*, 1952, 22 B, 206.

Euproctis melanopholis (Lepidoptera : Lymnatriidae) Affecting Livestock in Sudan

The occurrence of *Euproctis melanopholis* has not been previously reported from Sudan. These are widely distributed in Buram area, particularly during the wet season. It has been observed that milk contaminated with organism causes diarrhoea in man. Contaminated rain water causes diarrhoea to cattle and sheep. Even contaminated grass may lead to the same result. The hairs of the larva when blown onto human skin, cause an irritating rash. These observations agree with those reported from Europe and New England (U.S.) by Borrer and Delong (1963).

The author wish to express thanks to Under Secretary, Animal Resources, for permission to publish this note and the Director, Veterinary Research Administration for encouragement.

Veterinary Research Association, Ali I. Yagi.
Khartoum, Sudan, *September* 20, 1976.

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1. Borrer, J. Donaland. and De long, M. D., *An Introduction to the Study of Insects*, 1963, pp. 819.

Peronospora destructor (Berk.) Casp., A New Record from India

During May and June 1974–75, when the temperature was 50° F with high humidity, a violet coloured mildew growth on the entire leaf surface of *Allium cepa* L. (onion) plant was observed. The losses caused to the bulb and seed formation by the disease varied from 50 to 70%. The damage was more on the local as compared to exotic varieties. The plants were attacked at all stages except the seedlings. Affected plants were dwarfed, stunted, and pale green in colour. When expanding seed stems are infected, growth is checked by infection on one side of the stem, the latter bends in the direction of the lesion. As the seed umbel grows heavier, weakened stems break and seed maturation is prevented. The casual fungus was identified as *Peronospora destructor* (Berk.) Casp. which is a new report from India on onion.

Peronospora destructor (Berk.) Casp.

The fungus showed violet coloured mildew growth the entire surface of the leaf. On microscopic examination of the fungus, the sporangiophores were non-septate. Branching is monopodial with subacute to acute, sterigmata with a swollen base measuring 120–700 × 9·5–20 μ in dia. Conidia or sporangia are 18–29 × 40–68·8 μ in dia. with their base pointed and attached to sterigmata, thin walled, hyaline to subhyaline and slightly papillate at the distal end. No zoospores were observed.

The fungus tallies with the original description of Walker (1952) in most of the characters but for the abscence of zoospores.

Isolated from the leaves of *Allium cepa* L. KPAE Farm, Shalimar, Kashmir, specimen and slides deposited in *Herb. Cryptogammae Indiae Orientails,* Division of Mycology and Plant Pathoogy, IARI, New Delhi, as H.C.I.P. No. 32155.

Thanks are due to Dr. P. R. Mehta, BASF India Limitted, Prof. V. V. Chenulu, Dr. A. K. Sarbhoy, IARI, New Delhi, for confirmation of the fungus. Thanks to Director, Joint Director of Vegetables and Vegetable Specialist of J & K State for interest during the above study.

KPAE Farm, N. M. Mir.
Shalimar, Kashmir, *November* 13, 1976.

Ustilago vilfae Wint.—A New Record from India

Lesiurus sindicus L. locally called as 'Sewan' grass of desertic areas of Western Rajasthan was found to be heavily infected by a smut fungus during the year 1975–76. The grass is drought hardy and provides feed to the livestock for the mainstay of the local population. The protein contents of this grass in Jaisalmer and Bikaner districts varies from 7·0 to 9·81%. It is, therefore, considered king of desert grasses. Investigations of diseased plants revealed the presence of *Ust vilfae* Wint. The host and the pathogen are repo for the first time from India.

Ustilago vilfae Wint., *Bull. Torr. Bot.* C 1883, 10, 7.

Sori confined entirely in the inflorescence re and often completely destroying it. At first, i covered by a thin white peridium, hidden by enveloping leaf sheath which reptures, relea dusty black spore mass.

Spores in bunches or balls of five or more, yell ish brown to dark brown, globose to subglobo times ovoid 9·6–16·4 × 9·0–15·5 μ in length w 2–4 germ pores. Exospore wall yellowish reddish brown, verrucose to echinulate measur 1·0–1·25 μ in dia.

Specimen examined on graminaceous *Lesiurus sindicus* L. C.A.Z.R.I. Farm, Jaisalm Jodhpur (Raj.); Ahuja, L. D. 4-9-1976, H.C No. 32094.

The description of the fungus tallies with original description of Fischer (153)[1].

The authors are thankful to Professor V. Chenulu, Division of Mycology and Plant Pathol IARI, New Delhi, for providing necessary labor facilities.

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December 28, 1976.

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1. Fischer, G. W., *Manual of North Ame Smut Fungi,* The Ronald Press Comp N.Y., 1953, p. 343.

ERRATA

In the article entitled "Chromosome pairi the *Solanum surattense* × *S. melongena* F_1 he zygote" under Letters to the Editor in pages 125 of *Current Science,* Vol. 46 (4), 1977, following corrections may kindly be noted:

1. *S. surettense* should be read as *S. suratt*
2. "Ring of chain of" in Table I ma read as "Ring or chain of" and con to associations shown as V, IV & III.
3. The legend for Photomicrograph u Figure 2 may be read as "Metapha showing a chain of three (extreme ri ten bivalents and a univalent. heteromorphy of bivalent at left (arr

REVIEWS AND NOTICES OF BOOKS

Annual Review of Microbiology. (Vol. 30). Edited by Mortimer P. Starr, John L. Ingraham and Sidney Raffel. (Annual Reviews, Inc., Palo Alto, Calif.), 1976. Pp. 662. Price USA : $ 17.00 ; elsewhere : $ 17.50.

Every microbiologist gets the satisfaction that the publication of the Vol. 30 of *Annual Review of Microbiology* has "something for everybody." There are a number of articles which provoke interest in everyone; to quote a few—Genetic manipulation of microorganisms: Potential benefits and biohazards by Roy Curtiss III; The conquest of the major infectious diseases in the United State: A bicentennial retrospect by Rodney M. Wishnow and Jesse L. Steinfeld; Bacterial membrane structure by Milton R. J. Salton and Peter Owen. In the introductory autobiography, Dr. McElroy has very lucidly written the major influences on his scientific career and impressed upon us as to why a rich research environment is important in future career. The article "Endogenous viral enzymes involved in measenger RNA production" by Rajendra Raghow and David Kingsbury is a very useful survey. As mentioned in the introduction, this is the first review on the enzymes of RNA synthesis in viruses, and it has come at an appropriate time when voluminous amount of work was put, in this area during a short period, especially on the enzymes that modify 5′ termini of the viral messenger RNA.

A conventional but interesting area of work "Glycerol dissimilation and its regulation in bacteria" has been reviewed by E.C.C. Lin. A regrettable shortcoming in this article is, however, the lack of citation of recent references in certain areas. For instance, the author refers to a review article in Bacteriological Reviews of 1951 but ignores a mort recent review on the same subject in 1972. The hoped for, aim of this article is "to promote more studies on the diverse glycerol systems in a teleonomic context, as well as to summarize knowledge already gained,"

In the article on "Production of extracellular proteins by bacteria," Dr. Glenn reviews the subject of molecular biology of secretion. It is a well-organized, concise survey of the subject and includes all the speculative theories of exoprotein secretion. As mentioned in the introduction, the fascinating problem of the process of secretion has drawn the attention of scientists belonging to different disciplines and this review has very clearly presented to them, what the questions are which are not answered yet in the understanding of the synthesis and secretion of excproteins, especially questions regarding the specificity of exoprotein synthesis and involvement of discrete classes of membrane-bound ribosomes in the synthesis and secretion of exoproteins.

In the light of the recent concern among scientists and the public that exciting new genetic-engineering techniques pose potential biohazards, Roy Curtiss III, who is a member of the NIH recombinant DNA molecule programme advising committee, has done a commendable job in reviewing the entire area, thus giving a clear-cut view of the problems in the genetic engineering field in his review "Genetic manipulation of microorganisms: Potential benefits and Biohazards." After briefly considering the potential beneficial applications of recombinant DNA research, he has given a comprehensive account of the potential biohazards and various ways to contend with them.

M. S. Shaila.
T. Ramakrishnan.

Magnetism—Selected Topics. Edited by Simon Foner. (Gordon and Breach Science Publishers, New York), 1976, Pp. xx + 748, Price £ 44.00.

In *Magnetism-Selected Topics*, the editor, Dr. Simon Foner, has achieved what he had set out to accomplish. The resulting compilation is indeed an advanced text of current reference and source book for researchers actively engaged in this rapidly developing field of magnetism in the itinerant interacting electron systems—transition and rare-earth metals and magnetic alloys. The fifteen chapters (748 pages) are written by experts in the respective areas and cover almost every aspect of the problem. The first two chapters develop the basic theory in terms of the Stoner–unrestricted Hartree-Fock (time-dependent) picture. A most welcome feature of these, and in fact of all the subsequent Chapters, is the consistent effort to make the theory bear on the experimental findings. Chapter III discusses the non-uniform spin and charge responses. Chapter IV through IX are concerned with dilute (isolated magnetic impurity) and concentrated (interacting impurities alloys and nearly ferromagnetic systems and discuss in detail the transport properties of these systems and some many-body effects like the Kondo effect. Chapter VII on the local spin fluctuation, discusses in detail the formation of moment from a dynamical point of view. The rest of the book is devoted to the specific topics of current interest to such as hyperfine interaction in metal, photo-electron polarization, spin waves in insulators, neutron scattering, critical point phenomena, and cooperative effects in magnetic and biological system. The last mentioned is a new line of development in the spirit of cross-disciplinary endeavour. There are, however, some notable omissions. For example, one would have liked the elegant treatment of Kondo effect from the renormalization-group theoretical point of view to be included. There are minor typographical errors (*e.g.*, an annihilation operator is missing in Eq. 7 on p. 6). On the whole, one can say without reservation that the book will be a valuable addition to the Physics section of any Library.

N. KUMAR.

A Text Book of Algae. By N. D. Kamat. (S. Chand and Co., Ltd., New Delhi 110 055), First Edition. Pp. 1–162, 21·5 × 14 cms. Price Rs. Eight only.

As the author himself has indicated in the preface, this book has been written with the sole purpose of teaching Algae for the B.Sc. students of the Universities of India. Text books of this nature are welcome because they greatly help undergraduate teaching in the Indian Universities. After a brief introduction, the contents of the book are dealt under Procaryota—Cyanophyta, Eucaryophyta—Chlorophyta, Chrysophyta, Phaeophyta and Rhodophyta. Life histories of the types usually taught at the Indian Universities are described in detail in a fairly clear language, suitably illustrated by a number of line drawings. While most of the line drawings are adequate, it is seen at least some of them are very diagrammatic. This could be improved at least in the future edition. While describing the structure, modern concepts have also been included in simple language, even at the electron microscope level. This is certainly a redeeming feature, thus bringing the recent concepts on the structure of the algae. The errors in spelling of certain words in the body of the textbook could have been avoided. In general, it is a good attempt and is therefore recommended as a text for the B.Sc. students of the Universities of India.

K. SUBRAMANYAM.

Annual Review of Biochemistry. (Vol. 4 Edited by E. E. Snell, P. D. Boyer, A. Meist C. C. Richardson. (Annual Reviews, Inc., Cali U.S.A.), 1976. Pp. 1011. Price: USA $ 18.0 elsewhere $ 18.50.

The forty-fifth volume in this series contains reviews covering 920 pages with another 80 pa of indexing. Approximately 8,000 references ha been listed. The authors being different, readabil of the articles varies and some tended to be i specialized on a localized topic. This series alw gave the biochemists a chance to catch up with t developments in this fast-growing field. At prese the growth of the subject is so massive that ev these reviews cannot catch up. The covera has been limited to 30 topics by which not currently fascinating fields are covered, nor so of those covered fall into this category.

The topics can broadly be classified as prote (5), protein synthesis (3), enzymes and regulati (6), polyprenoids (3), nucleic acids (5) and b logical phenomena (8). No doubt each arti makes a good survey of the field—not limited admission of authors to the last year or few yea and summarize the developments in the opinion each author. These have attained a style quality over the past few years that these volum have become classics and are worthy of posses in all libraries that serve biochemists. The edit and authors of this volume also richly dese compliments. However, if the present method selection of topics continues, this series will beco an "annual collection of reviews in biochemi instead of "annual review of biochemistry".

One innovation, the editors have introduced this volume is the section called "Perspectives Summary" in which "the authors are invited locate their particular district on the biochem map....and to summarize......those devel ments they feel are most noteworthy". On scrutiny, most of these fall short of the designs when perfected they may become the only publi sections. On the other hand, if the major findi are itemized at the end, it may be equally usef

Looking purely from a localized angle, there nearly 100 Indian names in the index and of th hardly a handful represent work carried out in Indi laboratories. Many authors claimed that they m no attempts to catalogue all publications in the ti and have regretfully omitted several good pap It is somewhat painful to see several good obsrvati made in India, and known to this reviewer worthy, in a number of the 30 topics covered w ignored. The authors have certainly the right choose the papers. But the scanty coverage contributions from the developing countries needs to be taken note of.

T. RAMASARMA.

Prawns and Prawn Fisheries of India. By C. V. Kurian and V. O. Sebastian. (Hindustan Publishing Corporation, 6 U.B., Jawahar Nagar, Delhi-7), 1976. pp. xiii + 280, Price: Rs. 75.

In this compilation, the authors have attempted to provide all the information available on the prawns of India under one cover. The authors' concise writing and the many nice illustrations and diagrams make this book, one of good clarity. The text generally Includes the most recent scientific knowledge and the chapters on the fishery clearly coordinate the available information. The book has attempted to cover too many topics but has succeeded in avoiding overlaps. The authors have had to blend a text for academic and popular use and they have succeeded in doing this in an interesting informative and readable way for the most part but too brief in a few places.

This volume is a welcome addition in a field which is to-day gaining very great importance in seafood exports. It gives a careful, well-balanced, up-to-date account of prawn fishery in India. A chapter on the parasites and diseases of the prawns would have made this volume complete and more useful. The authors have concentrated only on the commercially important species but nevertheless, to justify the title, a detailed key on all the species of prawns of India would have greatly enhanced the value of this volume as an excellent reference source. The book can be recommended to all, and, contains much that will be of value to those interested in prawn fishery. The volume is reasonably priced and has a pleasant and durable appearance.

R. NATARAJAN.

Annual Review of Medicine : Selected Topics in the Clinical Sciences (Vol. 27). Editor : W. P. Cregor. (Annual Review, Inc., California), 1976. Pp. 530. Price : USA $ 17.00 ; Elsewhere : $ 17.50.

This volume presents highly informative review articles on selected topics in the clinical sciences. Included are chapters on cardiology, clinical pharmacology, endocrinology and metabolism, gastrointestinal tract, kidneys and urinary tract, neurology, psychiatry, haematology, neoplasia, infection and a few other miscellaneous topics.

The authors present an up-to-date information which along with the cited literature will serve as a useful reference source in the areas covered by the topics.

M. SIRSI.

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INTERNATIONAL SYMPOSIUM ON BIOMOLECULAR STRUCTURE, CONFORMATION, FUNCTION AND EVOLUTION

The Symposium will be held from January 4–7, 1978 at Madras. Professor Dorothy Hodgkin, Nobel Laureate, has consented to preside over the Symposium.

Scientific Programme: Original research papers are invited on topics relating to the title of the Symposium. A list of sub-topics is given below to serve as a guide-line on which papers may be contributed: 1. Protein folding, 2. Nucleic acid Conformations, 3. Polysaccharide Conformations, 4. Protein Nucleic acid interactions, 5. Structure-function relationships, 6. Protein Crystallography, 7. Biomolecular evolution, 8. Structure and Conformation of Medically important (and other) biomolecules, 9. Quantum biophysics, 10. Theoretical methods of Conformational analysis, 11. Experimental Study of Solution Conformations —N.M.R., O.R.D., I.R. and Laser Raman Spectroscopy, etc. 12. Biomolecular assembly and Supermolecular Structures, 13. Molecular basis of membrane phenomena, 14. Structure and Conformation of Membrane Components. 15. Molecular aspects of Drug-receptor and Enzyme-substrate interaction.

After the Symposium, it is planned to arrange a Winter School for a week from January 9–14, 1978 comprising of lectures by experts who will be attending the Symposium.

All correspondence connected with the Symposium may be addressed to the Convenor, Professor R. Srinivasan, Department of Physics (Crystallography and Biophysics), University of Madras, Guindy Campus, Madras 600 025.

ANNOUNCEMENTS

AWARD OF RESEARCH DEGREES

Utkal University has awarded the Ph.D. degree in Chemistry to Shri Panchanan Pujari.

Karnatak University, Dharwar, has awarded the Ph.D. degree in Chemistry to Shri Bheemarao Ganapatrao Ugarkar.

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degree in Chemistry to Shri P. Ramaswamy and Shri K. Venugopal Chetty.

Osmania University has awarded the Ph.D. degree in Physics to Shri A. Satyanarayana Murty; Ph.D. degree in Chemistry to Shri K. Rama Rao and Shri P. Hanumanthu, Ph.D. degree in Botany to Kum. A. Leelavathi.

M.S. University of Baroda has awarded the Ph.D. degree in Chemistry to Shri Joel Edwin Shah; Ph.D. degree in Microbiology to Smt. Anjana Jitendra Desai and Shri Harish Gulabrai Desai; Ph.D. degree in Geology to Shri Digamber Ramachander Ramachandra Gadekar.

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NICKEL(II) COMPLEXES OF 6-METHYLPYRIDINE-2-ALDOXIME

M. MOHAN, H. C. KHERA, S. G. MITTAL AND A. K. SIRIVASTAVA
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WE recently reported the far infrared and electronic spectra of mono- and *bis*-complexes of copper (II) with pyridine-2-aldoxime (HPOX) and HMPX, and discussed these data in terms of the electronic and geometric structures of the complexes[1]. The nickel (II) complexes studied have been mainly restricted to complexes of HPOX[2,3] HMPX[3] and 4, 6-dimethyl-*s*-triazine-2-aldoxime[3]. We have now carried out a detailed study of the nickel (II) complexes of HMPX.

Nickel (II), salts, sodium iodide (all reagent grade), 6-*m*-ethylpyridine-2-aldoxime (HMPX) were used without further purification. [Ni(HMPX)$_2$Cl$_2$] and [Ni(HMPX)$_2$Br$_2$] were prepared as described previously[3].

Diiodo-*bis* (6-methylpyridine-2-aldoxime) nickel (II) : Ni(HMPX)$_2$(NO$_3$)$_2$ was prepared by the quantitative treatment of Ni(NO$_3$)$_2$.6H$_2$O and HMPX in ethanol. A suspension of green crystals of Ni(HMPX)$_2$(NO$_3$)$_2$ (0·01 mol) in ethanol when treated with an ethanolic solution (0·02 mol) of NaI gave a greenish blue solid which was filtered, washed with water to remove NaNO$_3$, and finally with ethanol and then dried *in vacuo* over KOH.

Diffuse reflectance spectra at room temperature were recorded on a Cary Model 14-Spectrophotometer equipped with a diffuse reflectance accessory using magnesium oxide as reference. The infrared spectra of the ligand and its complexes in Nujoll mulls have been made on Perkin-Elmer infracord spectrophotometer. Magnetic measurements were obtained by the standard Gouy balance, calibrated with mercury tetrathiocyanatocobaltate (II). Metal and halogen analyses were by the authors and C, H and N were by Micro-analytical laboratory, I.I.T., Kanpur. Analytical data are given in Table I.

The magnetic susceptibilities (Table I) observed by these compounds are in good agreement with previous work and are consistent with those reported for distorted octahedral compounds[4].

The reflectance spectra of [Ni(HMPX)$_2$X$_2$] (X = Cl, Br or I) complexes exhibit the features associated with [Ni(en)$_2$Cl$_2$] (en = ethylenediamine) complex, which has shown to possess a dimeric chlorine-bridged structure in which the nickel (II) atoms tre in a *cis*-octahedral environment[5]. Therefore, the bands observed at *ca.* 9·7, *ca.* 10·6 and *ca.* 11·6 kK in [Ni(HMPX)$_2$X$_2$] can be assigned to the $^3A_2 \rightarrow {}^3B_1$, $^3A_2 \rightarrow {}^3B_2$ and $^3A_2 \rightarrow {}^3A_1$ transitions, all of which arises from the splitting of the ν_1 band (O_h symmetry) when the symmetry is lowered to $C_2{}^6{}_v$. The bands observed at *ca.* 15·6 and *ca.* 16·9 kK can be assigned to the $^3A_2 \rightarrow {}^3A_2$, $^3B_1$ and $^3A_2 \rightarrow {}^3B_2$ transitions, which arises from the ν_2 band (O_h symmetry). These assignments differ from those of Krause *et al.*[3], who assumed a *trans*-octahedral structure (D_{4h}) for these complexes. The transitions arising from the ν_3 band (O_h symmetry) are obscured by very intense charge transfer bands in the spectra of these complexes.

A partial assignment of the bands observed for HMPX and its nickel (II) complexes is given in Table II. The i.r. spectrum of HMPX differs from the conventional oximes, which shows a band at *ca.* 3250 cm^{-1}. This band is replaced by multiple bands between 3194 and 2791 cm^{-1}. This implies a much stronger hydrogen bonding than in other oxides. The strong bands observed at 1515 and 980 cm^{-1} in HMPX can be assigned to —C=N (acyclic) and N—O stretching vibration whereas the band observed at 820 cm^{-1} can be assigned to

TABLE I

Analytical and magnetic results

Compound	Calc. (%)					Found (%)					μ_{eff} (B.M.) at 298° K
	Metal	C	H	N	X	Metal	C	H	N	X	
Ni (C$_7$H$_8$N$_2$O)$_2$ Cl$_2$	14·62	41·8	4·52	14·0	17·6	15·10	42·12	4·70	14·31	17·91	3·19
Ni (C$_7$H$_8$N$_2$O)$_2$ Br$_2$	11·96	34·3	3·29	11·4	32·5	12·20	34·49	3·36	11·61	32·66	3·15
Ni (C$_7$H$_8$N$_2$O)$_2$ I$_2$	10·04	28·74	2·73	9·5	43·44	11·10	28·79	2·81	9·66	43·54	3·12

TABLE II

I.R. absorption bands due to $-C=N$, $N-O$, *and Ring Vibrations I – IV* (cm^{-1})

Compound	$-C=N$	$-N-O$	Ring I	Ring II	Ring III	Ring IV
HMPX	1515 (s)	980 (s)	1585 (s)	1564 (s)	1479 (m)	1436 (s)
$Ni(HMPX)_2Cl_2$	1632 (w, b)	1039 (s)	1612 (s)	1563 (w)	1477 (s)	1426 (m)
$Ni(HMPX)_2Br_2$	1629 (m)	1042 (s)	1595 (s)	1565 (w)	1490 (s)	1443 (m)
$Ni(HMPX)_2I_2$	1630 (sh)	1048 (s)	1598 (s)	1562 (w)	1482 (s)	1438 (w)

the out-of-plane wag of the hydrogen bonded oxime proton. Rings bands appear at 1585, 1564, 1479 and 1436 cm^{-1} in the free HMPX. However, the nickel (II) complexes exhibit the $-C=N$ band above 1600 cm^{-1} and $N-O$ band at *ca.* 1075 cm^{-1}. Similar band positions in the spectra of nickel, platinum and palladium complexes of HPOX, has previously been reported[7]. Therefore, these data indicate that oxime proton is not hydrolysed in $[Ni(HMPX)_2X_2]$ complexes and there is a contribution from the $-C=N-OH$ grouping in these complexes.

In the region 450–200 cm^{-1} HMPX exhibits absorption bands at 403s, 387m and 217m, whereas the nickel (II) complexes have absorption bands at *ca.* 425m, 417s, 344m, 321m, 278m and 230m cm^{-1} Since, there are no terminal Ni–X bands, no ν Ni–X vibrations will be expected above 200 cm^{-1}[8]. The relatively low frequencies assigned to these ν Ni–X vibrations would be consistent with a halogen bridging structure, although ν Ni–X strrtching freuuencies occur over a wide range and some terminal ν Ni–X frequencies have been reported. The very low-symmetry of these complexes should lead to the observations of upto four infrared active metal-nitrogen stretching vibrations. Two strong bands lie at *ca.* 350 cm^{-1} and 280 cm^{-1}, whilst a weaker band lies at *ca.* 300 cm^{-1}, these are therefore assigned as ν Ni–N vibrations.

In conclusion, reflectance and i.r. spectra indicate a *cis*-dimeric structure for $[Ni(HMPX)_2X_2]$ (X = Cl, Br or I). Dimeric structures of this type have been found to occur extensively in complexes of nickel (II) halides with ethylenediamine and related ligands.

The authors are thankful to Dr. P. C. Gupta, for providing necessary facilities in experimental work.

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1. Mohan, M., Khera, H. C., Mittal, S. G. and Sirivastava, A. K., *Acta Chim.* (In Press).
2. Krause, R. A. and Busch, D. H., *J. Amer. Chem. Soc.*, 1960, 82, 4830.
3. —, Guy, C. and Hooker, M. L., *Inorg. Chem.*, 1966, 5, 1825.
4. Lever, A. B. P., *Ibid.*, 1965, 4, 763.
5. Antsyshkina, A. S. and Porai-Koshita, M. A., *Doklady Akad. Nauk. S.S.S.R.*, 1962, 143, 105.
6. Sacconi, L., *Transition. Metal Chem.*, 1968, 4, 215.
7. Krause, R. A., Colthup, N. B. and Busch, D. H., *J. Phys. Chem.*, 1961, 65, 2216.
8. Postmus, C., Ferraro, J. R., Quattrochi, A., Shobatake, K. and Nakamoto, K., *Inorg. Chem.*, 1969, 8, 1851.

NANNOPLANKTON PRODUCTION IN VELLAR ESTUARY

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ABSTRACT

Nannoplankton and netplankton primary production were estimated at 3 stations in Vellar estuary during premonsoon and monsoon months of 1974. On the average nannoplankters were responsible for 71·1% of total production in the estuary. Irrespective of surface or bottom, the nannoplankters contributed between 40 and 100% (mean 64·4%) at Station I, 50 and 100% (mean 67·7%) at Station II and 26·8 and 100% (mean 80·7%) at Station III, to the total primary production. Salinity was the chief ecological factor that influenced nannoplankton production in the Vellar estuary.

INTRODUCTION

STUDIES made in various biotopes[1-12], showed that nannoplankton formed the major source of primary production. Nannoplankters play a significant part in marine as well as estuarine food chain; they form the main source of food for microzooplankton[13,14] and larvae of most of the benthic animals[15-17]. The present paper records variations in the nannoplankton primary production at 3 stations (Fig. 1) in the Vellar estuary, (mean depths 2·0, 2·5 and 2·75 m respectively) during the premonsoon (July–September) and monsoon (October–December) seasons of 1974.

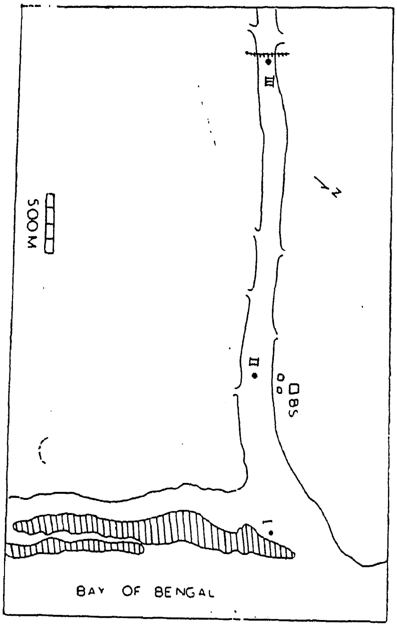

FIG. 1. Location of different stations at Vellar Estuary.

METHODS

All the samplings were carried out during high tides and surface samples were collected with a clean plastic bucket. Bottom samples were collected by means of horizontal water scoop. Light and dark bottle technique[18] was adopted to measure primary productivity and the samples were incubated *in situ* in the natural environment for 3 hrs. By adopting fractional filtration method, using a very fine mesh net (20 μ — Nytex), nannoplankton productivity was separately determined besides total productivity.

RESULTS AND DISSCUSSION

In the present study the range of variations in temperature were between 26·5 and 31·0° C, 26·0 and 30·0° C and, 25·0 and 28·0° C at stations 1, 2 and 3 respectively. The monthly variations in salinity and light penetration are shown in Table I. The etsimated nutrient concentrations (phosphate, ammonium, nitrite, nitrate and silicate) didn't exhibit much variation in the premonsoon months, but higher concentrations were observed during the monsoon months. At Station I, in the surface waters peak nannoplankton production was observed in July. It decreased in August followed by an increase in September (Fig. 2). After that the production decreased in the following months and attained the minimum in November. However, the production due to nannoplankton increased in December. The same type of distribution

TABLE I

Monthly variations in light, salinity and premonsoon—monsoonal variations in the net: nanno ratio

Month	Depth	Ex-coefficient			Salinity %			Season	Net: Nanno ratio			For whole estuary
		Station I	Station II	Station III	Station I	Station II	Station III		Station I	Station II	Station III	
July	Surface	2·30	2·40	1·70	33·0	30·0	29·0	Pre-monsoon	0·87	0·46	0·54	0·62
	Bottom				34·0	33·5	31·0					
August	Surface	2·50	2·80	1·90	34·5	33·5	31·0					
	Bottom				35·5	34·5	33·0					
September	Surface	1·60	2·15	5·50	15·5	13·0	22·0					
	Bottom				16·5	14·0	24·5					
October	Surface	1·85	2·60	3·50	20·5	17·5	11·5	Monsoon	0·47	0·42	0·48	0·46
	Bottom				24·0	19·5	12·5					
November	Surface	2·25	2·05	2·30	25·0	14·0	9·0					
	Bottom				27·5	24·5	22·5					
December	Surface	1·85	2·05	2·20	18·5	13·5	10·0					
	Bottom				25·0	18·0	11·0					

pattern was observed in the bottom waters also (Fig. 2). But in the case of netplankton production of the surface water, the pattern was reverse in the months of July, August and September whereas in the bottom it decreased gradually from the July maximum to the December minimum (Fig. 2).

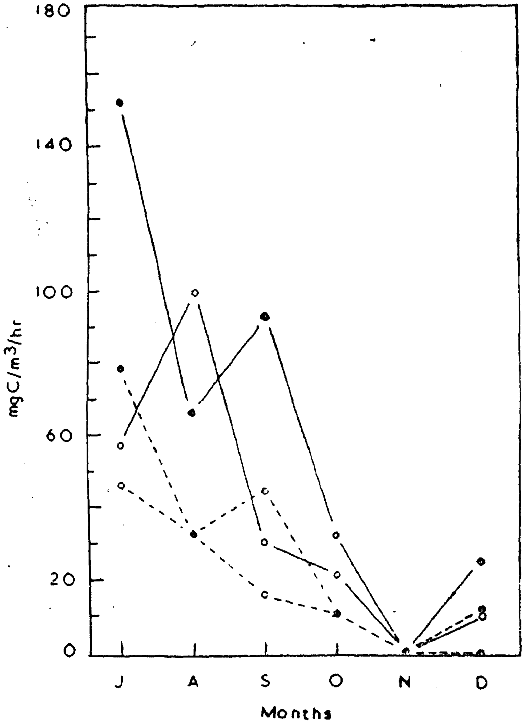

FIG. 2. Monthly variations in nanno and netplankton primary production at Station I.

At Station II, the variation in nannoplankton production of the surface water was similar to that observed at Station I. whereas in the bottom the peak production was in August unlike that of the surface waters (Fig. 3). With regard to the netplankton production in surface waters, the primary and secondary peaks were observed in the months of July and October respectively (Fig. 3). But in the bottom waters the peak value was recorded in August (Fig. 3).

At Station III, in the surface waters, the nannoplankton production increased and decreased alternately throughout the period (Fig. 4). Changes in the bottom waters were quite consistent with those of surface waters except for the decrease and increase in November and December respectively (Fig. 4). The netplankton production, in the surface decreased continually from July to November and then increased slightly in December (Fig. 4).

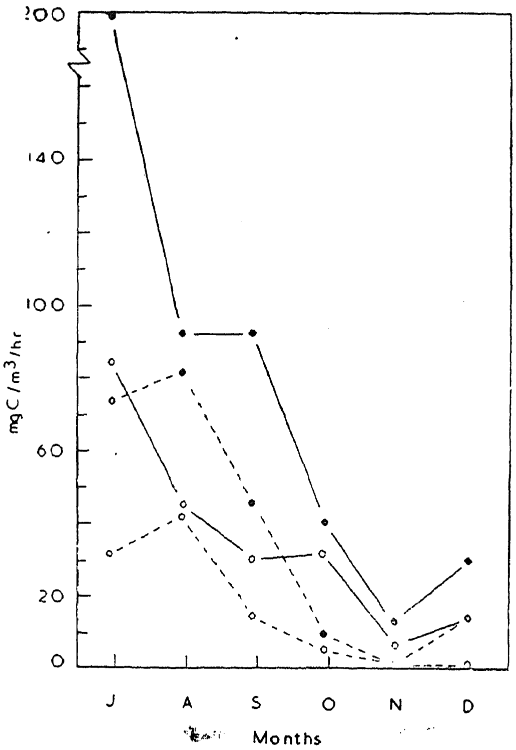

FIG. 3. Monthly variations in nanno and netplankton primary production at Station II.

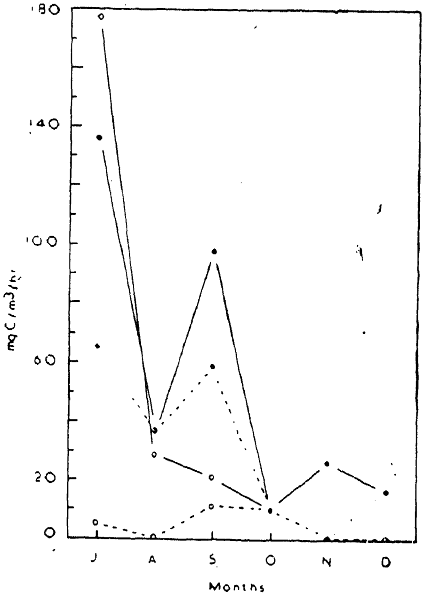

FIG. 4. Monthly variations in nanno and netplankton primary production at Station III.

●——● Nanno production in surface.
●- - - -● Nanno production in bottom.
O——O Netplankton production in surface.
O- - - -O Netplankton production in bottom.

Studies made so far in various neretic and oceanic waters, on nannoplankton primary production, indicated that nannoplankters contributed between 80 and 100% to total production[1,2,6-8]. In Brazilian mangroves, the range of nannoplankton production observed was from 64·3 to 95·8%[19] while in some eutrophic temperate lakes it was 90% and 50%[20,3]. In the mangrove and backwaters of Porto Novo, nannoplankton production was 80·5% and 62·1% respectively[21].

With regard to other estuaries, in the Chesapeake Bay estuaries, nannoplankters contributed between 89·6 and 93·4%[11] to the total plant production. In Cochin backwater's nannoplankton production was 74·47%[12]. The extent of nannoplankton production observed in the present study was more or less similar to that of the above observations. Among the three stations chosen in the Vellar estuary, Station II (Tidal zone, situated in the middle region of the estuary) showed higher rates of production due to nanno- as well as netplankton, during most of the months. Qasim *et al.*[22] pointed out that moderately low salinity would facilitate maximum production of most of the tropical phytoplankters. During the present study the salinity values observed in the tidal zone were consonant enough to promote larger plant production. Further as pointed out by Nash[23], the continually changing tidal direction in the estuary would have facilitated the phytoplankton forms to exist indefinitely within the middle region of the estuary thus contributing much to the greater productivity of that region. Although all the stations were shallow (Depth range 2·0 to 2·75 m) a definite decreasing gradient was obvious in nannoplankton productivity between surface and bottom waters (Figs. 2, 3 and 4). This might partly be due to the restricted sinking of the smaller cells having a high area/volume ratio as shown by Eppley *et al.*[24], and partly due to the aggregation of nannoflagellates towards the light saturated surface waters[25,26].

In the Vellar estuary, among the ecological factors considered, salinity appeared to be the most important factor in controlling the productivity of both nanno- and netplankton. Loftus *et al.*[26] pointed out that locally restricted heavy rainfall generally increased the rate of nannoplankton production in Chesapeake Bay estuarine waters. In the present study also a sudden decline in salinity due to local rainfall lead to an increase in nannoplankton production in all the 3 stations in the month of September (Figs. 2, 3 and 4). This may be attributed to dilution of estuarine water to a moderate salinity level which might have favoured the primary producers[22]. However, prolonged decrease in salinity during monsoon months lead to very low rates of production in all the 3 stations (Figs. 2, 3 and 4). This may be attributed to the washing away of the primary producers to the heretic region by the monsoonal flood. Though the rate of production decreased, the percentage contribution by nannoplankton increased and this was evident from the lower values of net : nannoplankton production ratio (Table 1) observed for the whole estuary.

ACKNOWLEDGEMENT

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1. Yentsch, C. S. and Ryther, J. H., *J. Cons. perm. Int. Explor. Mer.*, 1959, **24**, 231.
2. Anderson, G. C., *Limnol. Oceanogr.*, 1964, **9**, 284.
3. Kalff, J., *Ibid.*, 1972, **17** (5), 712.
4. Mommaerts, J. P., *Br. Phycol. J.*, 1973, **8**, 13.
5. Czeczuga, B., Gradeki, F. and Petrusewicz, K., *Bull. Acad. Pol. Sci. Ser. Sci. Biol.*, 1974, **22** (6), 399.
6. Teixeira, C., *Bolm. Inst. Oceanogr.*, 1963, **13**, 53.
7. Malone, T. C., *Limnol. Oceanogr.*, 1971 *a*, **16**, 633.
8. —, *Fish. Bull.*, 1971 *c*, **69**, 799.
9. Tundisi, J. G., In : *Fertility of the Sea*, Ed. by J. D. Costlow, 1971, **2**, 603.
10. —, *Contracoes Inst. Oceanogr. Univ. S. Paulo. ser. Ocean. Biol.*, 1970, **19**, 1.
11. McCarthy, J. J., Taylor, W. R. and Loftus, M. E., *Mar. Biol.*, 1974, **24** (1), 7.
12. Qasim, S. Z., Sumitra, V., Joseph, K. V. and Balachandran, V. K., *Ind. J. mar. Sci.*, 1974, **3** (2), 146.
13. Parsons, T. R. and LeBrasseur, R. J., In : *Marine Food Chains*, Ed. by J. H. Steele, 1970, p. 325.
14. Kristianzen, J., *Mitt. Int. Ver. Theor. Angrew. Limnol.*, 1971, **19**, 253.
15. Bruce, J. R., Knight, M. and Park, M. W., *J. mar. biol. Asso. U.K.*, 1940, **40**, 337.
16. Stickney, A. P., *Ecology*, 1946, **45** (2), 283.
17. Tanaka, Y., Seijin, I. and Kiyoshi, K., *Bull. Tokai. reg. Fish. Res. Lab.*, 1970, **63**, 79.
18. Strickland, J. D. H. and Parsons, T. R., "A practical hand book of sea water analysis," *Fish. Res. Board Can., Ottawa Bull.*, 1968, **167**, 311.
19. Teixeira, C., Tundisi, J. and Santoro, J., *Bolm. Inst. Oceanogr., S. Paulo*, 1967, **16** (1), 39.
20. Gelin, C., *Oikos*, 1971, **22** (2), 230.
21. Ramadhas, V., Subramanian, B. R. and Venugopalan, V. K., *Mahasagar*, 1975 (In press).
22. Qasim, S. Z., Bhattathiri, P. M. and Devassy, V. P., *Mar. Biol.*, 1971, **12**, 200.
23. Nash, C. B., *J. mar. Res.*, 1947, **6**, 147.
24. Eppley, R. W., Holmes, R. W. and Strickland, J. D. H., *J. exp. mar. biol. ecol.*, 1967, **1** (2), 191.
25. —, Holm-Hansen, O. and Strickland, J. D. H., *J. Phycol.*, 1968, **4** (4), 349.
26. Lotus, M. E., Subbarao, D. V. and Seliger, H. H., *Ches. Sci.*, **13** (4), 289.

UNDERSTANDING HUMAN SYSTEMS

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THROUGHOUT history, mankind has known of various ways to attain higher levels of awareness. Ever since language become the organ of consciousness ; man has attempted to reach greater heights of personal achievement both in the physical and mental fields. It is unique to him that man lives, unlike all other animals, in a threefold space ; physical, social and spiritual (cultural, if one objects to spiritual). Living in the human sense is much more than adaptation, survival and homeostasis. It is something broader and deeper. Creativity and self-realization are uniquely characteristic of man and might indeed be the ingredients of his future evolution. This is the central theme of a book* dealing, perhaps for the first time, with the problems of the evolution of man from a 'transdisciplinary' point of view. Edited by Erich Jantsch and Conrad Waddington, it includes contributions by eminent scientists, philosophers, sociologists, systems science specialists, all looking at human evolution in its totality. It outlines our understanding of the movement of human evolution from the physical, biological, and socio-biological to socio-cultural aspects. It deals, in addition to scientific questions, with existential ones, such as the meaning of life, specially relevant to man and his future.

ORGANIC EVOLUTION AND MAN

It is recognized that the origin of man marked a continuation and perhaps a culmination of the evolutionary processes which started long before man appeared on the scene, and it remains, not only with scientists but with philosophers and creative artists as well, an interesting inquiry as to the future of man and the processes through which this future is likely to be achieved. The well-known cliché that organic evolution through natural selection has now come to an end and that man's future evolution lies entirely in his culture is only partially true. That cultural communication of a unique kind is now available to him has to be granted. That evolution of the past which led to the creation of new species of animals should yield place to evolution of society and of the individual in man is also clear. But it is now being increasingly recognized that man still remains subject to the shaping influences of natural selection and his physical evolution has not, as some will argue, come to a halt. True enough, these influences are being reduced in some respects, as through medical technology ; it is also true that it is being intensified in others, as through pollution, disease and other man-made hazards. Waddington argues that to maintain that natural selection has become inoperative in relation to man is to create a too simplistic impression of the role of culture and the current human condition. It is more nearly a situation of *quid pro quo,* where, while certain selection pressures have been reduced or eliminated, others have been substituted. It is important to note that natural selection acts on phenotypes which become adapted during their lifetime by physiological processes to meet their particular stresses. Selection would act on populations subjected to these stresses and there is certain to be some genetic variation in the capacity of different individuals to respond to these stresses. Living organisms including man are open-ended, non-linear systems and an adaptive change originally induced by some environmental stress may, after continual selection over many generations, form part of the hereditary endowment of a later generation. Once we consider evolution in terms of selection of phenotypes, we find ourselves forced to conclude that in man, perhaps a little less than in sub-human animals, biological evolution is a matter of 'interlocking series of open-ended, cybernetic, circular processes'. While it is true that in lower animals, cultural transmission of information has played a relatively minor role, in man it is characterized by an enormously greater development of this type of sociogenetic mechanism operating through the agency of language. A qualitative difference does exist between human evolution and evolution of other animals but it does not seem to exclude natural selection altogether.

What is this cultural evolution ? What is the present state of the human system ? What are the possible directions in which this evolution may take place ? It takes more than a prophet to discern the lineaments of future man, but it should not be difficult to outline, on the basis of the past evolution of man and his present condition, the steps to be taken to secure a reasonable assurance of his future.

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* *Evolution and Consciousness : Human Systems in Transition* : Ed. Erich Jantsch and Conrad H. Waddington, Addison-Wesley Pub. Co., 1976, p. 359.

Waddington, Walter Pankow and Erich Jantsch, emphasise the openness of living systems, not merely the openness to flows of energy, matter and information but one which goes beyond it. The human world incorporates the basic principle of self-transcendence, of venturing out to change its physical, social and cultural conditions and especially to change its own consciousness. This continuous self-renewal and self-expression are unique to the human system. Any attempt to impose boundaries to this system becomes counter-productive. The human world is not one of equilibrium perfection, stability and predictability. Indeed, its present features as well as the possibilities of its future lie in its imperfection, non-equilibrium, non-predictability and symbiotic pluralism.

C. S. Holling provides evidence for this by his concept of 'continuity in change'. His studies of ecological systems have revealed that their dynamics cannot be explained by an equilibrium-centered view. Resilience, or capability to persist through changes, is linked to high fluctuations. Any attempt to stabilize an ecosystem ruins it. What evolution seems to do is not develop efficiency but flexibility to persist. High resilience, high fluctuations lead to and induce persistence.

ORDER THROUGH FLUCTUATION

Holling's premise seems naturally to lead to the most important chapter, that of Ilya Prigogine, who emphasises the non-equilibrium state as inherent in life at all levels. In a highly penetrating analysis of physical, chemical and biological systems, including that of Man, Prigogine develops his concept of 'Order through Fluctuation'. Fluctuations play a relatively minor role in classical physics. The whole domain of classical thermodynamics based on Boltzmann's Principle illustrates this. On the other hand, non-linear systems under non-equilibrium conditions bring about fluctuations which lead to new states. Indeed, 'dissipative structures' are themselves fluctuations which are continuously in exchange with their environment, and their evolution contains an essential stochastic element. Prigogine has developed new mathematical equations to deal with both deterministic as well as stochastic phenomena, This has become relevant in view of the wide gap that still separates physics and biology, between study of simple motions met with in rational mechanics and the very complex but orderly movements of chromosomes during mitosis or of cells during embryonic development. The idea of evolution in physics and that in biology are markedly in contrast. In physics, entropy marks a progressive disorganization of the system. In biology, it is accompanied by a progressive structuration culminating in the complicated organization of human society. Prigogine's is perhaps the first notable attempt towards a new mathematics, a 'bioalgebra', for studying living systems. And it now seems possible to go further and discuss more precisely the basic concepts in biology and sociology in mathematical terms as has already been done, for instance, by Volterra in ecology.

BIOLOGICAL SYSTEMS AUTOPOIETIC

Ralph Abraham discusses the principles and applications of macrodynamics in the light of recent developments, especially the theory of catastrophes developed by René Thom. A macron is a stable regime in a dynamic system and many examples abound in physical, chemical and electrical systems. Attempts to apply macrodynamics to biology, especially to neurophysiology have only just begun and are yet at a purely conjectural stage. On the other hand, biological systems are autopoietic, self-renewing, self-repairing and unity-maintaining, autonomous organizations. An animal cell is an example of a simple autopoietic system; and look at the number, dimensions and complexity of the chemical reactions going on in it at a given instant. What mathematics can adequately cover them? And how many thousands of differential equations are required to express them? An autopoietic system can never be understood by a study, however intense, of each component of the system. Its properties and functions can be comprehended only by an understanding of the *interactions* between the components, that is, by a study of its dynamical organization.

HUMAN SYSTEMS IN TRANSITION

This is complex, but the human organization is enormously more so. The complexity of structure and organization of modern human society exceeds that of any other biological system. It calls for a new mode of inquiry, beyond operations research, econometrics or applied mathematics. Human aspirations, human objectives, human ideals, are dynamic, complex, individual, and are constantly in conflict. And it is these very qualities that constitute the source of evolutionary unfolding of Man. Milan Zeleny and Norbert Pierre are forthright in their affirmation that human systems encompass the whole hierarchy of natural systems: physical, biological, social and spiritual. They are not amenable to methods of reduction. They are too complex for systems analysis or design. Their management is not cybernetics or information theory, or a theory of general equilibrium; optimal control theory or theory of conflict resolution does not explain them. They cannot be simplified by the rigour of mathematical mechanics. Electronic circuitry, communication channels, feedback loop mechanisms are no help in their understanding. They are organic, dynamic manifestations of organizational autopoiesis. They are self-renewing, self-repairing, self-realizing systems, with ability to anticipate the future, to formulate objectives, to plan their attainment and to make decisions. They deal with humans. Human systems management is not merely interdisciplinary or multidisciplinary. It is vastly more ; it transcends all disciplines.

Prigogine's principle of 'Order through Fluctuation' and the new field of non-equilibrium thermodynamics are as validly applicable to human society as to biological systems. We are moving towards a theory of self-organization of processes and structures, as Alistair Taylor points out in his essay. Our socio-cultural evolution is one of *transformation*. In the past, science and technology were the chief agents in changing the man–environment relationship. What is required in the future is a progressive reversal of these traditional roles. Science and technology must direct their attention to conserving the resources of the planet and managing our global ecology. At the same time new social institutions, new strategies and values in our cultures, are called for ; invention of new and appropriate techniques to unlock the enormous, hidden, and hitherto untapped, creative potential of the human individual as well as of cultures and social groups.

Marney and Schmidt's historical presentation of the evolution of the scientific method, beginning with the *axiomatic* prototype of Aristotle and Euclid down to Copernicus, through the *empirical* one of Newton, Maxwell and Einstein, to the *constructural* prototype of modern science, provides a perspective for the contemporary modification of the scientific method, which may be called the *normative* prototype. A gradual relinquishment of quest for certainty, continuing accretion of cognitive controls and a maximization of freedom and scope are characters of this new trend. These are the essentials of man's commitment to his future policy—a policy of conscious, purposeful alignment with the processes of emergence.

CRISIS AND CREATIVITY

No one knows the total potentialities of mankind. The transformation of human consciousness has passed through several levels, from cultural revitalization to creativity, outlined by O. W. Markely. In each case, a crisis in a human system calls for new ways of meeting it whether it is through revolution in science, heroic mythology or creativity. In each case, there is a transformation of consciousness, a METANOIA, leading to a new paradigm. Particularly important is the new emerging science dealing with consciousness that could contribute significantly to this new paradigm. Man has always striven towards attaining greater and higher levels of awareness, — of himself and of the external world, and of the relation between the two. He has not made much progress so far, certainly not in the West ; for he has all along felt that the new tools he had devised were adequate. Feedback training, learning theory, etc., have produced limited results. It would now appear that these methods need to be combined with the older traditional methods of the East, — Meditation, Yoga, Kundalini, Pránáyáma, — to unlock rapidly the untapped potentials of the human mind, especially its creativity, and powers of healing.

THE NEW IMAGE

Here is the evolving image of man. It involves the recognition of the identity between man and his world, between microcosmos and macrocosmos, between *atman,* the reality within, and *brahman,* the true essence of reality without. It consists of a perennial search for the self-realization of this ultimate relationship and its unfolding.

Historically, three modes of perception and inquiry have dominated man's attempts to recognize, establish and integrate this relationship between himself and the world around him. Jantsch calls them (*a*) mythological, (*b*) rational and (*c*) evolutionary. In the mythological, man enters into a relationship with God. The dominant factor is *Fear,* specially fear of falling from Divine Grace. In rational inquiry, illustrated by the methods of science, the subject and object are separated from each other and *Certainty* governs. In the third, the evolutionary inquiry, the dominant factor is *Hope* ; the subject and the object, *atman* and *brahman,* become integrated into a wholeness expressed in the Upanishadic *Tat Tvam Asi* (That Thou Art).

The future evolution of Man lies in a reestablishment of the three primal aspects of human unfolding, which the Chinese have called the Tao of the Earth, the Tao of Man and the Tao of Heaven. In Hindu terms, these rest in three primal values, *'Satyam, Sivam, Sundaram'.* The mythological and the rational inquiries have in the past made their contributions to human society and its evolution, but their relevance in the determination of the future image of man seems limited. It is the 'evolutionary' mode that holds hope for the future. Here is the great challenge : recognition of the totality of man and one-ness of the human race ; self-realization and development of the individual; a holistic perception of life and an appreciation of the true essence of humanness. These call for a new effort, *superconscious learning*, equivalent perhaps to our *Sujnana*, a 'consciousness to change consciousness', a self-reflective approach, providing a sense of direction for cultural processes and a dynamic guidance for mankind reaching out into the future. It calls for the development of a new understanding of human systems as an expression of life, going beyond adaptation and stabilization in order to develop a new paradigm of self-realization through self-transcendence, which is an integral aspect of evolution ensuring qualitative change,—a new science of humanity.

This is not easy and might well cause disruption. But in the words of A. N. Whitehead quoted by Jantsch at the head of his introductory chapter: 'It is the business of the future to be dangerous..... The major advances in civilization are processes that all but wreck the societies in which they occur'.

INSTITUTION OF CHEMISTS (INDIA), ASSOCIATESHIP EXAMINATION, 1977

The Twenty-eighth Associateship Examination of the Institution of Chemists (India) will be held in November, 1978. The last date for Registration is 30th November, 1977. The Examination in Group A (Analytical Chemistry) is divided into the following eleven sections and each candidate will be examined in two of them according to his choice as approved by the Council, in addition to the General Chemistry including Organic, Inorganic, Physical and Applied Analytical Chemistry: (1) Analysis of Minerals, Silicates, Ores and Alloys; (2) Analysis of Drugs and Pharmaceuticals; (3) Analysis of Foods; (4) Analysis of Water and Sewage; (5) Biochemical Analysis; (6) Analysis of Oils, Fats and Soaps; (7) Fuel and Gas Analysis; (8) Analysis of Soils and Fertilisers; (9) Analysis Connected with Forensic Chemistry; (10) Analysis Connected with Leather Chemistry; (11) Analysis Connected with Textile Chemistry; The Examination is recognised by the Government of India as equivalent to M.Sc. in Chemistry for purposes of recruitment of Chemists.

Further enquiries regarding this and for Membership may be made to the Honorary Secretary, Institution of Chemists (India), Chemical Department, Medical College, Calcutta-73.

INTERNATIONAL AWARD IN INDUSTRIAL HEALTH

Prof. S. H. Zaidi, Director, Industrial Toxicology Research Centre (ITRC), Lucknow, has been selected by the American Industrial Hygiene Association to receive Yant Memorial Award, for the year 1977, for his pioneering contribution in the field of environmental and occupational health. Professor Zaidi is the first Indian to receive this award in industrial health.

AWARD OF RESEARCH DEGREES

Utkal University, Bhubaneswar, has awarded the Ph.D. degree in Botany to (Miss) Bharati Behard.

Tamil Nadu Agricultural University, Coimbatore, has awarded the Ph.D. degree to Shri N. Natarajan and Sargunam Davis Peter.

M.S. University of Baroda has awarded the Ph.D. degree in Chemistry to Shri B. Vinayak Kamath and Shri George Kurian, V; Ph.D. degree in Biochemistry to Shri Vishnu Prakash; Ph.D. degree in Physics to Shri Rameshchandra Thakorlal Shah.

Karnatak University, Dharwar, has awarded the Ph.D. degree in Mathematics to Shri Kulkarni Krishnaji Hanumantharao.

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degrees to Shri V. Sundara Raja (Physico) and to Shri P. Satyan (Zoology).

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degree in Zoology to Shri A. V. Ramana Dikshitulu.

LETTERS TO THE EDITOR

ROLE OF THREE PHONON NORMAL PROCESSES IN THE PHONON CONDUCTIVITY OF SILICON

IN SPITE OF the fact that some refinements[1-4] have been proposed recently, the success of the Callaway[5] theory of the lattice thermal conductivity in explaining the experimental results has been tremendous at low temperatures. Using the displaced Planck distribution function, Callaway obtained an expression for the lattice thermal conductivity which can be divided into two parts. The first part gives the lattice thermal conductivity due to the combined scattering relaxation rate while the second part is known as the correction term due to three phonon normal processes. In the recent studies[6-10], it has been found that the contribution of the correction term due to three phonon normal processes towards the total phonon conductivity can be neglected due to its very small contribution except for solid He[11] and LiF[12]. Recently Dubey and Verma[6] studied the role of three phonon normal processes in the phonon conductivity of Ge and it is reported that the Callaway integral gives best result for $\tau_N^{-1} < \tau_R^{-1}$ and for the relative scattering strength of three phonon normal and umklapp processes as 10^{-3}. Following the earlier work of Dubey and Verma, in the present note, we have studied the role of three phonon normal processes in the lattice thermal conductivity of Si in the temperature range 2–100° K and it is found that the Callaway integral of the lattice thermal conductivity gives best fit between calculated and experimental value of the phonon conductivity for the relative scattering strength of three phonon normal and umklapp processes 'a' $= 10^{-3}$. It has also been reported that for $\tau_N^{-1} > \tau_R^{-1}$, the Callaway integral of the lattice thermal conductivity not only fails to give good fit but also leads to a wrong temperature dependence of the phonon conductivity which is similar to the previous report of Dubey and Verma for Ge.

Following the earlier work of Dubey and Verma and considering the Callaway theory, the lattice thermal conductivity of an insulator can be expressed as:

$$K = (k_B/2\pi^2)\ (k_B T/\hbar)^3\ (1/v_s) \int_0^{\theta/T} \frac{x^1 e^x (e^x - 1)^{-2}\, dx}{v_s/L + Dx^1 + E_1 x^2/(1+a)} \quad \text{for } \tau_N^{-1} < \tau_R^{-1}$$

and for $\pi_N^{-1} > \tau_R^{-1}$ it reduces to

$$K = (k_B/2\pi^3)\ (k_B T/\hbar)^3\ (1/v_s) \left\{ \frac{\left[\int_0^{\theta/T} x^4 e^x (e^x - 1)^{-2}\, dx\right]^2}{\left[\int_0^{\theta/T} \left(v_s/L + Dx^4 + E_1 x^2/(1+a)\right) x^4 e^x (e^x - 1)^{-2}\, dx\right]} \right\}$$

where k_B is the Boltzmann constant, $\hbar$ is the Planck constant divided by 2π, v_s is the average phonon velocity, L is the Casimir length of the crystal, θ is the Debye temperature and E_1 is the three phonon scattering strength. In writing the above stated expression, following scattering relaxation rates have been used.

Boundary scattering relaxation rate [13] $\tau_B^{-1} = v_s/L$

Point defect scattering relaxation rate [14] $\tau_{pt}^{-1} = Aw^4$

Three phonon normal processes scattering relaxation rate $\tau_N^{-1} = B_2\, w^2\, T^3$

Three phonon umklapp processes scattering relaxation rate $\tau_U^{-1} = B_1\, w^2\, T^3$

Combined three phonon scattering relaxation rate $\tau_{3ph}^{-1} = (B_1 + B_2)\, w^2 T^3 = E\, w^2 T^3 \equiv E_1 x^2$

where B_1 and B_2 are the scattering strengths of three phonon umklapp and normal processes respectively and A is the point defect scattering strength. In the expressions (1) and (2), 'a' means B_2/B_1 and $\tau_R^{-1} = \tau_B^{-1} + \tau_{pt}^{-1} + \tau_U^{-1}$.

The values of the different parameters which have been used in the calculation of the phonon conductivity of Si in the temperature range 2–100° K are listed in Table I. To study the role of normal processes, lattice thermal conductivity has been calculated for the different values of the relative scattering strength of three phonon normal and umklapp processes, *i.e.*, $a = 10^{-3}$ to 10^3.

TABLE I

The parameters and constants used in the calculation of lattice thermal conductivity of Si to study the role of three phonon normal processes

$\tau_B^{-1} = 2 \cdot 0 \times 10^6$ sec^{-1}

$A = 2 \cdot 47 \times 10^{-45}$ sec^3

$E = 3 \cdot 4 \times 10^{-24}$ sec deg^{-3}

$v_s = 6 \cdot 53 \times 10^5$ cm/sec

$\theta = 658°$ K.

For $\tau_N^{-1} < \tau_R^{-1}$, eq. (1) is used to calculate phonon conductivity for the four different values of '*a*' (0·001, 0·01, 0·1 and 1·0). Similarly for $\tau_N^{-1} > \tau_R^{-1}$, lattice thermal conductivity is calculated for '*a*' = 10, 100, 1,000 using eq. (2). The result is shown in Fig. 1. From Fig. 1, it can be seen that the Callaway theory gives excellent agreement between calculated and experimental results of phonon conductivity of Si for $\tau_N^{-1} < \tau_R^{-1}$ when '*a*' is approaching to 10^{-3}. However for $\tau_N^{-1} > \tau_R^{-1}$, the theory fails to yield the correct temperature dependence of the phonon conductivity.

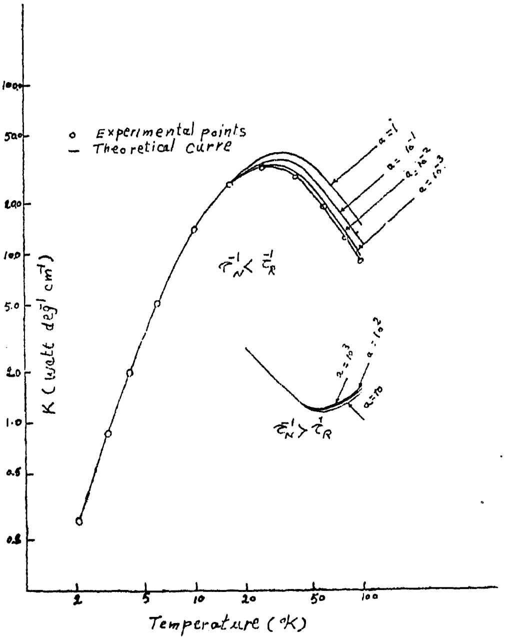

FIG. 1. Phonon conductlvity of Si in the temperature range 2–100° K. Solid lines are the calculated value. Circles are the experimental points. '*a*' is the relative scattering strength of three phonon normal and umklapp processes.

A Similar result was also reported by Dubey and Verma[6] for Ge in the temperature range 2–100° K. Thus, one can say that the role of three phonon normal processes is similar in the phonon conductivity of Si and Ge at low temperatures.

The authors wish to express their thanks to Prof. G. S. Verma for his interest in the present work.

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1. Holland, M. G., *Phys. Rev.*, 1963, 132, 2461.
2. Joshi, Y. P. and Verma, G. S., *Ibid.*, 1970, 1 B, 750.
3. Sharma, P. C., Dubey, K. S. and Verma, G. S., *Ibid.*, 1971, 4 B, 1306.
4. Dubey, K. S. and Misho, R. H., *Ibid.*, 1976, (To be published).
5. Callaway, J., *Ibid.*, 1959, 113, 1046.
6. Dubey, K. S. and Verma, G. S., *J. Phys. Soc. (Japan)*, 1972, 32, 1202.
7. —, *Ind. Jour. Pure & Appl. Phys.*, 1973, 11, 265.
8. —, *J. de Physique*, 1976, 37, 267.
9. Joshi, Y. P., Tiwari, M. D. and Verma, G. S., *Physica*, 1970, 47, 213.
10. Dubey, K. S., *J. de Physique*, 1976 (Communicated).
11. Agrawal, B. K. and Verma, G. S., *Phys. Rev.*, 1962, 128, 603.
12. Callaway, J. and Baeyer, M. C. V., *Ibid.*, 1960, 120, 1149.
13. Casimir, H. B. G., *Physica*, 1938, 5, 595.
14. Klemens, P. G., *Solid State Physics*, 1958, 7, 1.

CRYSTAL STRUCTURE OF METAMIZOL

1-PHENYL-2, 3-dimethyl-5-pyrazolone-4-methylamino-methane-sulphonate sodium, $C_{13}H_{16}N_3O_4S$. Na, known variously as metamizol, methampyrazone or novalgin, is a well known pain relieving drug. Here we give a preliminary account of the x-ray analysis of this compound carried out as part of a programme of structural studies on analgesics and their interactions.

The crystals were grown by the slow diffusion of ethanol vapour into an aqueous solution of the compound. The space group and the unit cell dimensions of the crystals were determined from oscillation, Weissenberg and Precession photographs, and the density was measured by flotation in a mixture of benzene and carbon tetrachloride. The density indicated the presence of one water molecule per formula unit. The cell dimensions were subsequently refined on a four circle diffractometer.

Crystal data : Space group $P2_1/c$.

$a = 9 \cdot 143\,(3)$, $b = 49 \cdot 50\,(2)$, $c = 7 \cdot 314\,(2)$ Å; $\beta = 90 \cdot 2\,(1)°$; $D = 1 \cdot 388 \pm 0 \cdot 005$. $D_c = 1 \cdot 409$ gm/c.c., $Z = 8$.

The intensity data were collected on a Picker four circle diffractometer. The structure was solved by the direct methods and refined isotropically to an

R value of 0·17. The positions of the crystallographically independent atoms in the structure are showing in Fig. 1.

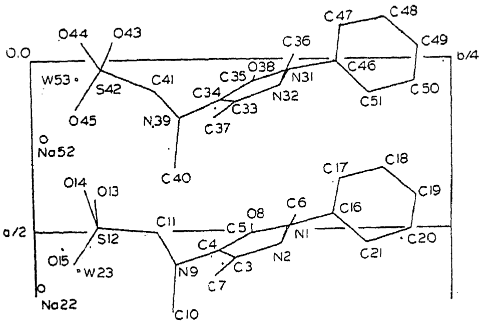

FIG. 1. Positions of the crystallographically independent atoms in the structure as viewed along the *c*-axis.

The dimensions of the two crystallographically independent molecules in the structure are nearly the same. The hetero nitrogen atoms in the five membered ring of each molecule are pyramidal with the attached phenyl and methyl groups lying on the opposite sides of the plane of the ring. The bond lengths and angles in the pyrazolone group are comparable to those observed in the other two analgetic pyrazolone derivatives, namely, antipyrine and amidopyrine[1,2]. The sodium ions in the structure are coordinated by sulphonyl oxygen atoms and water molecules. It is interesting to note that the carbonyl oxygen atom in the pyrazolone group is not involved in metal coordination or hydrogen bonding.

Further refinement of the structure is in progress.

The authors thank Professor G. N. Ramachandran, for his interest in the work. They are obliged to Mr. T. N. Bhat for many discussions, and Mr. F. Hansen and Dr. S. Larsen for help in data collection. The diffractometer was made available by the Danish Science Research Council. Financial support from the University Grants Commission, India, is gratefully acknowledged.

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1. Singh. T. P. and Vijayan, M., *Acta Cryst.*, 1973, 29 B, 714.
2. — and —, *Ibid.*, 1976, 32 B, 2432.

STABILITY CONSTANTS OF BIVALENT METAL CHELATES OF 2-(N-α-METHYL, 2-HYDROXY BENZYLIDENEIMINO) ETHANESULPHONIC ACID

HOLM *et al.*[1], and others[2] have recently reviewed the metal chelates of Schiff bases. The Schiff base 2-(N-α-methyl, 2-hydroxybenylideneimino) ethanesulphonic acid (H_2NE) was synthesized by the method reported[3] earlier by the condensation of ortho hydroxyacetophenone and 2-amino ethanesulphonic acid. H_2NE provides three possible coordination sites, *viz.*, $-SO_3H$, $-OH$ and $=N-$groups. The present investigation is concerned with the determination of stability constants of the chelates of Cu(II), UO_2(II) and VO(II) with H_2NE.

All titrations were carried out at 25° C, 30° C and 35° C by applying Calvin's extension of Bjerrum's method[4]. The following solution mixtures (total volume 40 ml) were prepared and titrated against 0·2 M potassium hydroxide: (A) 10·0 ml 0·02 M H_2NE + 4·0 ml. 1·0 M $NaClO_4$ + 26·0 ml water, (B) 10·0 ml 0·02 M H_2NE + 10·0 ml 0·02 M metal-ion solution + 4·0 ml 1·0 M $NaClO_4$ + 16·0 ml. water, (C) 10·0 ml 0·02 M H_2NE + 5·0 ml 0·02 M metal-ion solution + 4·0 ml 1·0 M $NaClO_4$ + 21·0 ml. water.

RESULTS AND DISCUSSION

Metal-H_2NE system.—The appearance of inflections after the addition of 1 and 2 moles of alkali/mole of ligand corresponds respectively to the neutralisation of $-SO_3H$ and $-OH$ groups. The reactions may be stated as:

$$H_2NE \rightleftharpoons |HNE|^- + H^+ ; \quad (1) \qquad pK_1 = 8{\cdot}08,\ 7{\cdot}99 \text{ and } 7{\cdot}91 \text{ at } 25°,\ 30° \text{ and } 35° \text{ C.}$$

$$|HNE|^- \rightleftharpoons |NE|^{--} + H^+ \quad (2) \qquad pK_2 = 9{\cdot}89,\ 9{\cdot}85 \text{ and } 9{\cdot}77 \text{ at } 25°,\ 30° \text{ and } 35° \text{ C.}$$

When equimolar concentration of the metal-ion is added, the shape of the free ligand titration curve changes appreciably as a result of chelate formation and two inflections at 2 and 3 moles of alkali/mole of ligands are indicated. The reactions may be represented as:

$$M^{2+} + H_2NE + 2\,OH^- \rightarrow (MNE)^0 + 2\,H_2O \qquad (3)$$

$$(MNE)^0 + OH^- \rightarrow \tfrac{1}{2}[M(NE)_2]^{2-} + \tfrac{1}{2}[M(OH)_2]^0 \qquad (4)$$

When the mixture containing metal and ligand in the ratio of 1 : 2 (taking half the concentration of the metal-ion), was titrated against standard alkali, two inflections at 1 and 2 moles of alkali/mole of ligand are observed which may be attributed

TABLE I

Stability constants bivalent metal chelates of H_2NE ($\mu = 1\cdot0$ M $NaClO_4$)

Metal-chelate		Stability constant 25°C	Stability constant 30°C	Stability constant 35°C	$-\Delta G^\circ$K.cals/mole 25°C	$-\Delta G^\circ$K.cals/mole 30°C	$-\Delta G^\circ$K.cals/mole 35°C
Cu (II)	log K_1	11·401 (0·001)	11·421 (0·001)	11·452 (0·002)	20·79	21·20	21·64
	log K_2	3·851 (0·001)	3·871 (0·001)	3·902 (0·002)			
UO_2 (II)	log K_1	11·601 (0·001)	11·842 (0·002)	12·102 (0·002)	21·27	21·93	22·62
	log K_2	4·001 (0·001)	3·971 (0·001)	3·952) (0·002)			
VO (II)	log K_1	12·101 (0·001)	12·232 (0·002)	12·352 (0·002)	22·37	22·88	23·40
	log K_2	4·301 (0·001)	4·271 (0·001)	4·251 (0·001)			

The deviations in the experimental and computational values are shown in parenthesis.

to the stepwise formation of 1 : 1 and 1 : 2 species as shown by the equations :

$$\tfrac{1}{2}\,M^{2+} + H_2NE + OH^- \rightarrow \tfrac{1}{2}\,[MNE]^0 + \tfrac{1}{2}\,H_2NE + H_2O \qquad (5)$$

$$\tfrac{1}{2}\,(MNE)^0 + \tfrac{1}{2}\,H_2NE + OH^- \rightarrow \tfrac{1}{2}\,[M(NE)_2]^{2-} + H_2O \qquad (6)$$

The above sets of titrations were also performed at 30° and 35° C and values of log K_1 and log K_2 were obtained. Different methods[5] were used to compute the stability constants and the values so obtained were found in agreement. The theoretical formation curves of these metal chelates at 25° C were derived by using average log K_1 and log K_2 values, as, 11·40 and 3·85 for Cu(II) chelate; 11·60 and 4·00 for UO_2(II) chelate; and 12·10 and 4·30 for VO(II) chelate, respectively. A perusal of the experimental and theoretical curves indicates negligible deviations. The experimental stabilities and their deviation from computational values along with the free energy changes are shown in Table I. The stability constants are in the sequence VO(II) > UO_2(II) > Cu(II).

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1. Holm, R. H., Everette, G. W. (Jr.), and Chakravorty, A., *Prog. Inorg. Chem.*, 1966, 7, 83.
2. Lyapina, S. S., Mishchenko, V. V., Trailina, E. P., Savich, I. A. and Spitsym, V. I., *Dokl. Akad. Nauk. SSSR*, 1970, **190**, 593.
3. Mehta, R. K. and Gupta, R. K., *Indian J. Chem.*, 1972, 11, 56.
4. Calvin, M. and Wilson, R. W., *J. Am. Chem. Soc.*, 1945, 67, 2003.
5. Irving, H. and Rossotti, H. S., *J. Chem., Soc.* (London), 1953, p. 3397.

ESTRAGOL—A PERSISTENT DEPOLARISING AGENT OF THE SKELETAL MUSCLE MEMBRANE

THE effect of Estragol, (Fig. 1, inner square) a substance isolated from the essential oil of Feronia Limonia was studied on the isolated sartorius semitendinosus and rectus abdominis muscles of frog (*Rana tigrina*). The muscles were suspended in an isolated organ bath containing aerated frog Ringer solution[1] and Isotonic contractions were recorded with gimble lever giving a 12 fold magnification.

Estragol caused contractions of the sartorius and the semitendinosus muscles when added in a concentration of 3 × 10^{-3} M. Initial contraction was followed by relaxation, even when the drug was present in the bath (Fig. 1, *a* and *b*). It caused contracture of the rectus abdominis of frog when added in the same concentration. This effect was markedly reduced in a K^+ (9 × 10^{-2} M)[2] depolarised muscle (Fig. 1, *a*) and in the presence of quinidine (5·1 × 10^{-4} M, 10 mts.) or Mn^{++} (1 × 10^{-2} M, 10 min.). It reduced the responses to K^+ (4 × 10^{-2} M, 2 mts.) on isolated frog rectus muscle (Fig. 1, *c*), The nature of the effect of Estragol on sartorius and semitendinosus muscles, reduction of its effect in K^+ depolarised muscle and in the presence of quinidine or Mn^{++3} and its ability to reduce the action of K^+ indicate that it produces its effect by persistent depolarisation of the muscle membrane. This effect is interesting, since it is produced by a substance having a chemical structure that is different from the known membrane depolarising agents[4].

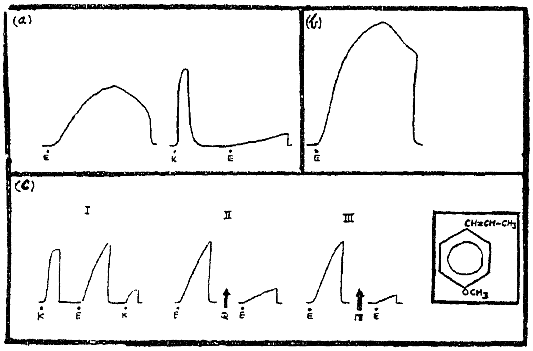

FIG. 1. (*a*) Isolated Sartorius muscle of frog (*Rana tigrina*). Effect of Estragol (at E, 3×10^{-3} M, 5 mts.) in normal and potassium depolarised (at K 9×10^{-2} M) muscle. (*b*) Effect of Estragol (at E) on Isolated Semitendinosus muscle of frog. (*c*) Isolated Rectus abdominis of frog—I. Effect of Estragol (at E) on potassium induced (at K 4×10^{-2} M, 2 mts.) contractures. II and III. Effect of quinidine (at Q, $5 \cdot 1 \times 10^{-4}$ M, 10 mts.) and managanese (at M, 1×10^{-2} M, 10 mts.) on Estragol (at E) induced contractures.

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1. *Staff Department of Pharmacology,* University of Edinburg, E and S, Livingstone, Ltd., London, 1968, p. 2.
2. Evans, D. H. L., Schild, H. O. and Theselff, H. L., *J. Physiol.* (*London*), 1958, 143, 474.
3. Chiarandini, D. J. and Stefani, E., *Ibid.*, 1973, 232, 129.
4. Koelle, G. B., *The Pharmacological Basis of Therapeutics,* 3rd Edition, Edited by Goodman, L. S. and Gillman, A, The Macmillan, Co., New York, p. 598.

EFFECT OF CAFFEINE ON TESTICULAR HYALURONIDASE OF DIFFERENT SPECIES

HYALURONIDASE is widespread in the semen of mammals and it has been suggested that the enzyme may play a role in the passage of spermatozoa through the zona pellucida[1, 2] by modifying the structure of this membrane to make it more susceptible to the action of another sperm-borne enzyme acrosin which is widely believed to be involved in the penetration process[3, 4]. The hyaluronidase activity of semen sample correlates with the number of sperm[5] and hyaluronidase in seminal plasma has been shown to originate from sperm acrosome[6]. Various authors have reported an increase in fertilizing capacity of spermatozoa on addition of this enzyme[7,8]. The present communication describes the *in vitro* effect of caffeine on testicular hyaluronidase of different species.

For standard hyaluronidase assay weighed testicular tissues from rat and goat were homogenized (1:9 w/v) in ice-cold 0·1 M sodium-phosphate citrate buffer pH 4·5. Cetyltrimethyl-ammonium bromide was added to a final concentration of 0·1% prior to homogenization in order to release the enzyme from its particulate bound form.[9] The homogenate was allowed to stand in ice for 30 minutes and was then centrifuged at 105,000 xg for 60 minutes at 4° in a VAC 60 ultracentrifuge. The clear supernatant obtained was used as the enzyme source. Human semen was frozen for 30 minutes and seminal plasma was removed as supernatant by centrifugation at 2,000 xg for 15 minutes[10] at 4°. The spermatozoa were washed and homogenized in 0·1 M sodium-phosphate citrate buffer, pH 4·5, as detailed above. Ovine testicular hyaluronidase was obtained from V.P. Chest Institute, New Delhi.

Hyaluronidase activity was assayed by the rate of release of free N-acetylglucosamine from hyaluronic acid by a modification of the colorimetric methods of Reissig *et al.*[11] and Bollet *et al.*[12]. Protein concentration was determined by the method of Lowry *et al.*[13] using crystalline bovine albumin as the standard.

Table I depicts the *in vitro* effect of 6·5 mmol/l caffeine on testicular hyaluronidase activity. With the exception of acrosomal hyaluronidase from human semen caffeine activates rat, goat and ovine testicular enzyme. Acrosomal enzyme was moderately inhibited to the extent of 14%. The order of hyaluronidase activation was

Ovine testis > goat testis > rat testis.

An increase in substrate concentration did not produce any change in the extent of caffeine inhibition or activation on hyaluronidase activity thereby suggesting that it probably acts by attaching to some allosteric site on the enzyme protein. Recently rabbit and bull acrosomal hyaluronidases have been shown to possess properties similar to the testicular enzyme[14].

TABLE I

in vitro *comparison of the effect of caffeine* (6·5 mmol/l) *on testicular hyaluronidase activity*

Species	Origin	% inhibiton (↓) or activation (↑) (mean ± S.D.)
Rat	testis	21·87±1·55 ↑
Goat	testis	51·65±0·84 ↑
Ovine	testis	235·76±0·02 ↑
Human	acrosome	14·38±0·66 ↓

Specific activity = μ moles of N-acetyl glucosamine liberated/mg. protein/minute.

In contrast to the above report the present study indicates that human acrosomal hyaluronidase appears to be different from rat, goat and ovine testicular enzyme with respect to its inhibition by caffeine.

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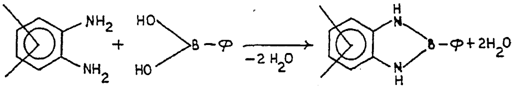
1. Stambaugh, R. and Buckley. J., *J. Reprod. Fertil.*, 1969, **19**, 423.
2. Hartree, E. F., *Of Microbes and Life*, eds., J. Monod and E. Borek, Columbia University Press, New York, 1971, p. 271.
3. Stambaugh, R., Brackett, B. G. and Mastroianni, L., *Biol. Reprod.*, 1969, **1**, 223.
4. Zaneveld, L. J. D., Robertson, R. T. and Williams W. L., *FEBS Letters*, 1970, **11**, 345.
5. Werthessen. N. T., Berman S., Greenberg, B. E. and Gargill, S. L., *J. Urol.*, 1945, **54**, 565.
6. Swyer G. I. M., *Biochem., J.*, 1947, **41**. 413.
7. Rowlands, I. W., *Nature*, 1944, **154**, 332.
8. Kurzrok, R., Leonard, S. and Conrad, H., *Amer. J. Med.*, 1946, **1**, 491.
9. Kumar Parveen, *M.Sc. Thesis*, Panjab University, 1972.
10. Roussel, J. D. and Stallcup, O. T., *J. Reprod. Fertil.*, 1966, **12**, 423.
11. Reissig, J. L., Strominger, J. L. and Leloir, L. F., *J. Biol. Chem.*, 1955, **217**, 959.
12. Bollet, A. J., Bonner, W. M. Jr. and Nance, J. L., *Ibid.*, 1963, **238**, 3522.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. K. and Randall, R. J., *Ibid.*, 1951, **193**, 265.
14. Zaneveld, L. J. D., Polakoski, K. L. and Schumaçher, G. F. B., *Ibid.*, 1973, **248**, 564.

SYNTHESIS OF SOME NEW BENZODIAZABOROLE DERIVATIVES

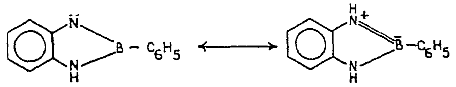
BENZODIAZABOROLE, pyrimidine and a few purine type boron analogues where $-\overset{|}{\underset{|}{C}}-H$ group in heterocyclic ring is replaced by boron were prepared and screened for treatment of cancer[1,2] with certain success.

Liao *et al.*,[3] have prepared and tested heterocyclic boronic acids for the same purpose. The application of boron heterocyclics as stabilizers, antimetabolite, disinfectants and chemosterilants have also been recorded.

In view of this, a number of benzodiazaboroles have been prepared. Their structures have been confirmed by elemental analyses and recorded I.R. frequencies. Heterocyclic boron compounds reported herein have been prepared by conventional methods, *i.e.*, azeotropic distillation of the reactants to remove the water formed in the reaction.



Their physical properties have been recorded in Table I. These compounds are iso-electronic with indole and show only a small absorption due to a dipolar B–N, B–O or B–S bonds. Non ionic form is the predominant of the two resonating structures.



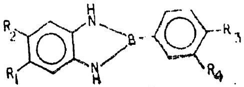
Preparation of Borimidazolines :

Equimolar amounts of the aryl boronic acid (0·01 mol) and *o*-phenylenediamine (0·01 mol) were taken in xylene and heated under reflux for 4-5 hours. The water was removed azeotropically and the solvent xylene was distilled off at reduced pressure. The products were crystallized from carbon tetrachloride or benzene.

The infrared spectra of these borimidazoline compounds show the presence of the important functional groups. All show the characteristic NH–streching absorption in the regions of 3450 — 3400 cm^{-1}, aromatic –CH– at 2950 — 2900 cm^{-1} and B–N group, being double bond character appears in the region of 1520 — 1380 cm^{-1}. The absorptions for N-H bands in KBr are very strong and sharp at 3470 cm^{-1} and 1430 cm^{1}, trivalent bornon at 1350 cm^{-1} and out of plane C–H modes of boron substituted aromatic rings at 760–750 cm^{-1}, 700-690 cm^{-1},

TABLE I

Borimidazolines



Sl. No.	R_1	R_2	R_3	R_4	M.P.°C	Yield per cent	Properties	Formula	N%		B%	
									Found	Reqd.	Found	Reqd.
1.	CH_3	H	H	H	223–224	80·7	light reddish brown flakes	$C_{13}H_{13}N_2B$	13·08	13·46	5·01	5·28
2.	CH_3	H	CH_3	H	229–231	72·9	light brown flakes	$C_{14}H_{15}N_2B$	12·54	12·61	4·83	4·95
3.	CH_3	H	OCH_3	H	> 293	67·6	greyish brown powder	$C_{14}H_{15}N_2OB$	11·58	11·76	4·65	4·62
4.	CH_3	H	Br	H	217–218	65·5	reddish brown plates	$C_{13}H_{12}N_2BBr$	9·93	9·76	3·80	3·83
5.	CH_3	H	H	NO_2	276–277	59·6	dark brown powder	$C_{13}H_{12}N_3BO_2$	16·83	16·60	4·52	4·35
6.	CH_3	CH_3	H	H	242–243	77·4	pinkish white shining plates	$C_{14}H_{15}N_2B$	12·78	12·61	4·83	4·95
7.	CH_3	CH_3	CH_3	H	257–258	80·1	pinkish brown shining crystals	$C_{15}H_{17}N_2B$	12·01	11·86	4·39	4·66
8.	CH_3	CH_3	OCH_3	H	138–140	62·3	dark brown crystals	$C_{15}H_{17}N_2OB$	11·04	11·11	4·18	4·37
9.	CH_3	CH_3	Br	H	231–233	67·4	light reddish brown shining crystals	$C_{14}H_{14}N_2BBr$	9·49	9·30	3·59	3·65
10.	CH_3	CH_3	H	NO_2	> 290	66·6	light brown flakes	$C_{14}H_{14}N_3BO_2$	15·64	15·73	4·23	4·12

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1. Nyilas, E. and Soloway, A. H., *Jour. Amer. Chem. Soc.*, 1959, **81**, 2681.
2. Brown, H. C. and Gupta, S. K., *Ibid.*, 1971, **93**, 1816; *J. Appl. Chem.*, 1965, **15**, 372.
3. Liao, T. K., Podrebarac, E. G. and Cheng, C. C., *J. Amer. Chem. Soc.*, 1964, **86**, 1869.

BEHAVIOUR OF *MACROPHOMINA PHASEOLI* AND *SCLEROTIUM ROLFSII* WITH RELATION TO SOIL TEXTURE AND SOIL pH

THE importance of edaphic factors on the disease incidence in the case of soil-borne plant pathogens has been duly emphasised[1,4] and the effect of soil temperature, moisture and organic matter on the growth and multiplication of *Macrophomina phaseoli* (Maubl.) Ashby and *Sclerotium rolfsii* Sacc. in soils free from plant debris has been noted[3,5,6], but it is not known whether soil texture and pH have any influence on these fungi under similar condition. This communication therefore attempts to present some information regarding the effect of soil texture and soil pH on the prevalence of *M. phaseoli* and *S. rolfsii* in soils which do not contain any fragments of plant tissues.

Air-dried field soil was passed through a 80-mesh sieve to remove all the sand particles and the percentage of silt and clay content was determined by mechanical analysis[7]. Four different textural groups were artificially made with incorporation of sand, silt and clay, *i.e.*, light soil (sand : silt : clay = 55 : 30 : 15), medium soil (40 : 40 : 20), medium heavy soil (25 : 50 : 25) and heavy soil (10 : 60 : 30) and pH of the soil was adjusted to different levels, *viz.*, pH 5, 6, 7 and 8 following the methods as described by Chattopadhyay and Bhattacharjya[2]. Soils were inoculated separately with *M. phaseoli* and *S. rolfsii* isolated from jute and wheat plants respectively having sterilized them by autoclaving. Both the sets were incubated under controlled condition in darkness at 27° C and 50% saturation level. Observations were taken at regular intervals and data recorded after plating in peptone-dextrose agar. Results representing an average of three replications are presented in Tables I and II.

The growth behaviours of *M. phaseoli* and *S. rolfsii* have been observed to be different under various conditions of the soil. *M. phaseoli* showed a marked increase in population at medium soil texture (sandy loam) and more or less at all pH levels followed by gradual decline. Both pH 5 and 6 were found to be effective for the fungus; whereas at pH 8, there was a steady fall. *S. rolfsii* showed similar behaviour in all the treatments though there might have been slight rise in population at the intermediate stage in light and medium type soils as well as in soils having pH between 6–7, but its presence (mycelial form) in soil could not be detected after 20 days of inoculation.

Pycnospores, chlamydospores and sclerotia are reproductive structures of *M. phaseoli* whereas *S. rolfsii* perpetuates in the form of only sclerotia. Although both the fungi show certain

TABLE I

Effect of soil texture on the prevalence of the pathogens

Count intervals (days)	Population (× 10^4)/gm of soil on dry wt. basis							
	M. phaseoli				*S. rolfsii*			
	Light soil	Medium soil	Medium heavy soil	Heavy soil	Light soil	Medium soil	Medium heavy soil	Heavy soil
0	1·28	1·24	1·54	1·43	0·66	0·62	0·73	0·66
2	1·46	1·39	1·32	1·10	0·47	0·44	0·36	0·25
4	1·24	1·83	1·11	0·77	0·51	0·47	0·36	0·22
7	0·73	1·61	0·77	0·58	0·18	0·14	0·03	0·03
12	0·88	1·02	0·73	0·47	0·03	0·00	0·00	0·00
20	0·88	0·80	0·51	0·44	0·00	0·00	0·00	0·00
Mean	1·02	1·31	0·99	0·79	0·30	0·27	0·24	0·19
	SEm ± 0·093	CD at 5%	0·2802		SEm ± 0·036	CD at 5%	0·1084	

TABLE II

Effect of soil pH on the prevalence of the pathogens

Count intervals (days)	Population (× 10^4)/gm of soil on dry wt. basis							
	M. phaseoli				*S. rolfsii*			
	pH 5	pH 6	pH 7	pH 8	pH 5	pH 6	pH 7	pH 8
0	1·46	1·39	1·50	1·54	0·77	0·69	0·73	0·73
2	1·32	1·61	1·57	1·39	0·55	0·58	0·51	0·44
4	1·65	1·54	1·06	1·17	0·47	0·66	0·51	0·33
7	0·95	0·95	1·02	0·99	0·29	0·40	0·33	0·18
12	0·84	0·88	0·73	0·51	0·03	0·14	0·07	0·11
20	0·80	0·88	0·66	0·44	0·00	0·00	0·00	0·00
Mean	1·17	1·20	1·09	1·00	0·35	0·41	0·35	0·29
	SEm ± 0·063	CD at 5%	0·1898		SEm ± 0·0219	CD at 5% 0·0659		

similarity in the production of sclerotia but they differ in their mycelial characteristics. In *M. phaseoli*, the mycelium is coarse, dark coloured and thick walled; but in *S. rolfsii*, it is fine, hyaline and thin walled. The differential behaviours of these two fungi in response to soil pH and soil texture may be due to the differences in the morphology of the mycelium.

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1. Baker, K. F., *Symposium on Soil Microbiology and Root-Disease Fungi—in Plant Pathology Problem and Progress 1908–1958*, by C. S. Holton, Univ. Wisconsin Press, 1959, p. 309.
2. Chattopadhyay, S. B. and Bhattacharjya, S. K., *Indian J. Agric. Sci.*, 1968, **38**, 176.
3. — and Mustafee, T. P., Unpublished record, 1969.
4. Garrett, S. D., *Biol. Rev.*, 1950, **25**, 220.
5. Mustafee, T. P. and Chattopadhyay, S. B., *Indian J. Microbiol.*, 1971 *a*, **11** (4), 77.
6. — and —, *Ibid.*, 1971 *b*, **11** (4), 83.
7. Piper, C. S., *Soil and Plant Analysis*, Hans Publishers, Bombay, India, 1966.

INHIBITORY EFFECT OF LIGHT ON THE PRODUCTION OF CITRIC ACID BY *ASPERGILLUS NIGER*

INFLUENCE of light of the visible range on growth, sporulation, etc., in microorganisms had been reported earlier[1-3]. Kamal *et al.*[4] had shown that light is necessary for good growth and formation of conidiophores, sterigmata, etc., in *Aspergillus niveus*. Little attention, however, was given on the effect of light on biosynthesis and accumulation of citric acid by fungal organisms.

Materials and Methods

The strain used for this study was *Aspergillus niger* 6N3 isolated from the soil of Naihati, West Bengal. Erlenmeyer flasks (100 ml) containing 25 ml of Shu and Johnson's medium (Shu and Johnson, 1948)[5] were inoculated with 0·1 ml of the conidial suspension (10 × 10^6 conidia/ml) and incubated at 30° C under light, darkness and alternate light and dark conditions. Cultures under light condition were kept in an incubator fitted with fluorescent lamps for continuous illumination. The intensity of illumination falling on the cultures was measured with a lux meter and the value adjusted at 2,200 lux units equivalent to 205 candle power approximately. The dark condition was created inside a chamber covered with black cloth. Alternate light and dark conditions were given to two sets of flasks in the following way:

1. 12 hrs. darkness followed by 12 hrs. light (Dark-Light).
2. 12 hrs. light followed by 12 hrs. darkness (Light-Dark).

Control cultures were kept in an incubator at 30° C, and opened only for casual observation. Observations were made on the 9th day of incubation. Mycelial dry weight was taken on previously weighed filter papers by drying them at 60° C for 24 hours. Total acidity was estimated by titrating the culture filtrates against 0·1 N NaOH solution to the phenolphthalein end point. Citric acid content was estimated following the methods of Marrier and Boulet (1958)[6]. Different organic acids accumulated in the culture filtrates were extracted in ether and detected by the thin layer chromatographic methods[7].

Results and Discussion

Mycelial dry weight remained almost the same in the cultures grown under the different conditions. This clearly indicates that light or dark condition is not having any remarkable effect on the vegetative growth of mycelium. Even though the total acid production had not varied much under light or dark conditions when given separately, it showed a slight increase when light and dark conditions were given alternately. A profound decrease in the citric acid production had been noticed in those cultures grown under light condition, while dark condition slightly increased the citric acid production (Fig. 1). Cultures grown under illumination

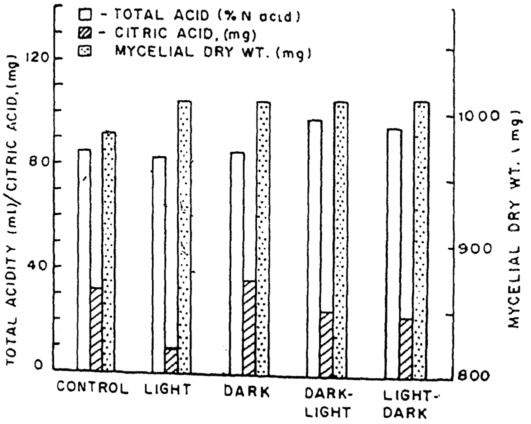

FIG. 1. Effect of light, dark and alternate light and dark conditions on mycelial growth and acid production of *A. niger* 6N3.

had shown the accumulation of glycolic and α-ketoglutaric acids in addition to the other acids (Table I).

TABLE I

Different organic acids accumulated in the culture filtrates of cultures grown under light, dark and alternate and dark conditions for 9 days at 30° C

Control (Normal incubation)	Light	Dark	Light-dark	Dark-light
Oxalic	Oxalic		Oxalic	Oxalic
Citric	Citric	Citric	Citric	Citric
Isocitric	Isocitric	..	..	..
	Succibic	Succinic	Succinic	Succinic
	α-Ketoglutaric			
	Glycolic			
		Malic		

Legend:
Light-dark: Light treatment followed by dark treatment.
Dark-light: Dark treatment followed by light treatment.

It has been found that light plays a significant role on the biosynthesis and accumulation of citric acid in the culture of *A. niger* 6N3. Generally, the synthesis and accumulation of citric acid occurs in mold cultures through the Krebs tricarboxylic acid cycle[8,9,10]. Presence of succinic, α-ketoglutanic, isocitric and glycolic acids in the culture filtrate of cultures grown under light condition probably suggest the presence of some alternate pathways like S.K.I. cycle[2] (Succinate-α-ketoglutarate-isocitrate). Therefore, the low citric acid yield may be due to depletion of the intermediates of TCA cycle to the S.K.I. cycle, necessary for the normal functioning of the TCA cycle. Production of citric acid by fermentation can be kept more or less constant if the fermentation is carried out in the dark as there should be very little chance under such a condition for a deviation in the nromal pathway through which citric acid is produced.

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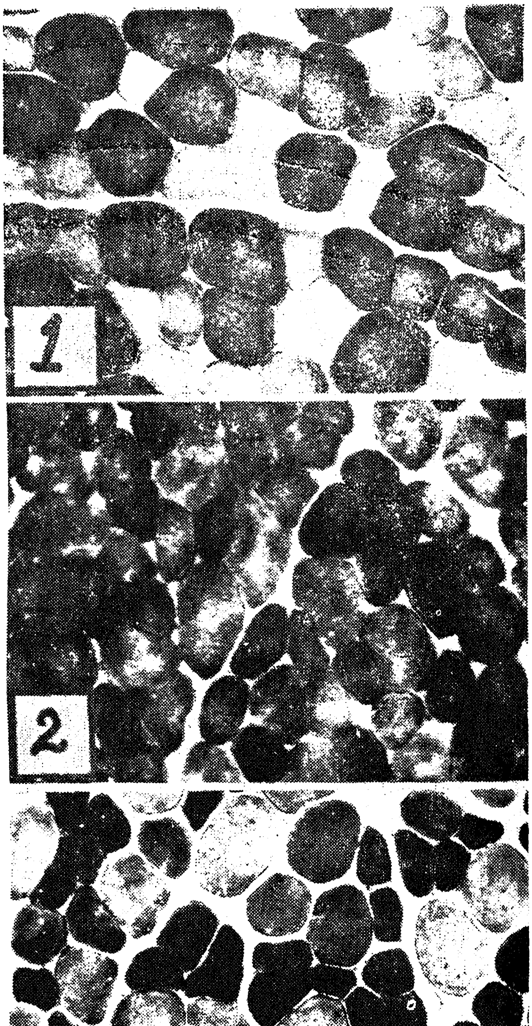
1. Huskins, R. H. and Weston, W. H. (Jr.), *Am. J. Bot.*, 1950, 37, 739.
2. Cantino, E. C. and Horenstein, E. A., *Mycologia*, 1956, 48, 777.
3. Neergaard, P. and Newhall, A. G., *Phytopathology*, 1951, 41, 1021.
4. Kamal, Gupta, M. L. and Nath, V., *Sci. and Cult.*, 1976, 42, 163.
5. Shu, P. and Johnson, M. J., *Ind. Eng. Chem.*, 1948, 40, 1202.
6. Marrier, J. R. and Boulet, M., *J. Dairy Sci.*, 1958, 41, 1683.
7. Meyers, W. E. and Ken-Yen-Huang, *Anal Biochem.*, 1966, 17, 210.
8. Lewis, R. F. and Weinhouse, S., *J. Am. Chem. Soc.*, 1951, 73, 2500.
9. Ramakrishnan, C. V., Steel, R. and Lentz, C. P., *Arch. Biochem. and Biophys.*, 155, 55, 270.
10. Abraham, T. K. and Chaudhuri, K. L., *Association of Microbiologists of India*, XVI Annual Conference, Abstracts No. 509, 1975, p. 28.

REEVALUATION OF α-GLYCEROPHOSPHATE DEHYDROGENASE ACTIVITY IN SKELETAL MUSCLE FIBRE TYPES

CONFLICTING reports have appeared in the literature about the histochemical localization and distribution of lactate dehydrogenase (LDH) and NAD⁺-linked α-glycerophosphate dehydrogenase (GPD). When assayed *in vitro* white muscle fibres show higher GPD activity, but show lower enzyme activity than the red muscle fibres in histochemical tests[1-4]. It has since been demonstrated, particularly for LDH[6], that when a sequential staining technique involving the use of phenazine methosulfate (PMS) is used, the white muscle fibres show higher enzyme activity than the red ones. This has led to the suggestion that PMS should be used for the histochemical demonstration of dehydrogenases's activity in locations where diaphorases are a limiting factor[3-10].

It has also been demonstrated[5] that LDH and possibly GPD also survive formalin fixation, provided the muscle sections have been fixed in formalin vapour. This step is important since without prior fixation, considerable leakage of these two dehydrogenases may take place particularly from white muscle fibres[1,5]. Therefore, using PMS (0·4 mg per ml of the incubation medium[5]) and formalin vapour fixation of the muscle sections, we have reevaluated the histochemical profile of GPD activity in mixed skeletal muscle fibre types. The diaphragm of the rhesus monkey (*Macaca mulatta*) was used in the present study. Formalin vapour fixed sections, 10 μ thick, were processed for the histochemical demonstration of GPD activity[11]. By varying the concentration of PMS in the incubation medium it was possible to elicit varying histochemical profiles, with higher or



Figs. 1–3. Fig. 1. Histochemical localization of GPD activity in the cross-section of monkey diaphragm using excess PMS in the incubation medium. Note darkly stained white and intermediate fibre types and unstained red fibres, × 50. Fig. 2. Same as in Fig. 1, with lesser concentration of PMS in the incubation medium. Note comparatively darker staining of the smaller red fibres as compared to the larger white fibres. × 50. Fig. 3. T.S. of monkey diaphragm, stained for lipids with Sudan Black B for the identification of the three basic muscle fibre types. Note the strong, medium and least staining for lipids in the red, intermediate and the white fibres respectively, × 50.

lower GPD activity in the white, red and intermediate muscle fibre types (Figs. 1, 2). When excess of PMS (higher than 0·4 mg/ml) is used in the incubation medium, there is suppression of the enzyme activity in the red fibres and a considerably higher activity could be elicited in the white fibres (Fig. 1). Monkey diaphragm served as a useful material for the present study since it contains all the three histochemically demonstrable muscle fibre types (Fig. 3). A more correct profile for GPD activity may be demonstrated in the various fibre types by employing the sequential staining technique (producing a combination of Figs. 1 and 2) as suggested earlier for the correct localization of LDH activity in mixed muscles[5].

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---

1 Kumar, P., *Ph.D. Thesis*, University of Delhi, 1974.
2. Blanchaer, M. C., Van Wijhe, M. and Mozersky, D., *J. Histochem. Cytochem.*, 1963, **11**, 500.
3. Van Wijhe, M., Blanchaer, M. C. and Jacyk, W. R., *Ibid.*, 1963, **11**, 505.
4. Fahimi, H. D. and Karnovskv, M. J., *J. Cell Biol.*, 1966, **29**, 113.
5. Talesara, C. L., Kumar, P. and Mohini, I., *Acta Histochem.*, 1973, **45**, 292.
6. George, J. C., Susheela, A. K. and Vallyathan, N. V., *J. Anim. Morph. Physiol.*, 1963, **10**, 24.
7. Farber, E. and Louviere, C. D., *J. Histochem. Cytochem.*, 1956, **4**, 347.
8. Fahimi, H. D. and Amarsingham, C. R., *J. Cell Biol.*, 1964, **22**, 29.
9. Conklin, J. L., *Stain Tech.*, 1966, **41**, 105.
10. Mathisen, J. S. and Mellgren, S. I., *J. Histochem Cytochem.*, 1965, **13**, 296.
11. Pearse, A. G. E., *Histochemistry: Theoretical and Applied*, 2nd Edn., J. and A. Churchill Ltd., London, 1961.

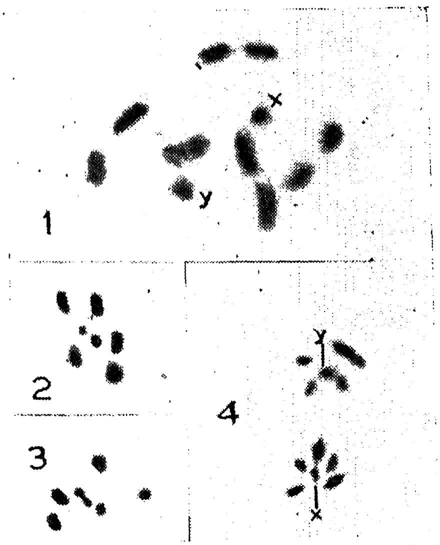
THE CHROMOSOMES OF *COPTOSOMA INDICA* MONTAND (HETEROPTERA—PLATASPIDAE)

Among Heteroptera the cytology of the family Plataspidae has received little attention. The species worked out are *Brachyplatys subaeneus, Coptosoma biguttula, Coptosoma* sp., *C. punctatissimum, C. cribrarium, C. variegata* and *C. scutellatum*[1-5]. The cytological data suggest that the modal number of chromosomes in the family is 12 $(10 + X + Y)^{1-5}$. The present study is based on an examination of

50 specimens of *Coptosoma indica* collected from Kakatiya University campus. No deviations from the family karyotype have been observed.

The collection of male specimens was made from the host plant *Tephrosia hirta*, and *T. hamiltonii* during September and October, 1976. The testes were dissected and fixed in Acetic Alcohol (1 : 3). Each testis consists of seven compartments held together throughout the length by a surrounding thick sheath. The observations reported here are from temporary as well as permanent haematoxylin squash preparations of testes[6].

Spermatogonial metaphase cells (Fig. 1) could be identified by the presence of 10 autosomes and XY and sex chromosomes. At metaphase I (Fig. 2) the two univalent sex chromosomes often lie at the centre surrounded by 5 autosomal bivalents. In metaphase II (Fig. 3) they form a pseudo-bivalent. The behaviour of the chromosomes during anaphase II (Fig. 4) is quite normal with respect to the equal distribution of autosomes and XY segregation to opposite poles.



FIGS. 1–4. Haematoxylin squashes of the testes of *Coptosoma indica*, × *ca*, 2,600. Fig. 1. Spermatogonial metaphase. Fig. 2. Metaphase I. Fig. 3. Metaphase II. Fig. 4. Anaphase II.

These findings indicate that a comparison of chromosome morphology with other known species[1-5] may be of some value in determining interrelationship within the family Plataspidae. In recent years many chromosome banding techniques have been devised to establish phylogenetic relationship among eukaryotes. Sumner *et al.*[7], described a simple and elegant technique for Giemsa banding. Heteropteran insects have not been studied extensively for banding patterns. Most of them possess small and holokinetic chromosomes. Giemsa banding of metaphase chromosomes has been described in Heteropteran insects *Triatoma infestans* and *Rhodnius prolixus*[8]. Similar studies could be of significance in elucidating the chromosomal affinities in the family Plataspidae and other members of Heteroptera.

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---

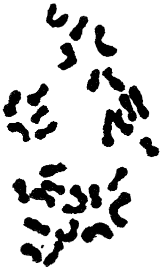
1. Banerjee, M. R., *Proc. Zool. Soc.* (Calcutta), 1958, 11, 9.
2. Takenouchi, Y. and Muramoto, N., *Jour. Hokkaido Gakugei Univ.* (Section II B), 1967, 18, 1.
3. Makino, S., *An Atlas of the Chromosome Numbers in Animals*, Iowa State College Press, Ames., Iowa, 1951.
4. Jande, S. S., *Res. Bull.* (N.S.), Punjab Univ., 1959, 10, 215.
5. Mikolajski, M., *Experientia*, 1968, 24, 1062.
6. Subramanyam, S. and Subramanyam, M. K., *Proc. Ind. Acad. Sci.*, 1970, 72 B, 115.
7. Sumner, A., Evans, H. J. and Buckland, R., *Nature*, 1971, 232, 31.
8. Maudlin, I., *Ibid.*, 1974, 252, 392.

A FERTILE COLCHICINE INDUCED ALLOHEXAPLOID IN *ARACHIS*

A STERILE triploid interspecific hybrid between cultivated groundnut (*Arachis hypogaea*, $2n = 40$) and a wild annual diploid (*A. duranensis*, $2n = 20$) was obtained by Seetharam *et al.*[3], and the cytological analysis of this hybrid revealed the allopolyploid nature of groundnut with one genome common between *A. hypogaea* and *A. duranensis*. This cross was mainly attempted by them to transfer the genes for tolerance to tiyka leaf spot from *A. duranensis* to *A. hypogaea*.

The failure of pod set in the triploid was due to high pollen sterility (82·5%) as against 5·0% in the parents[4]. With a view to inducing fertility in this sterile hybrid, amphidiploids were induced by subjecting its vegetative cuttings to colchicine treatment. Twentyfive stem cuttings of four inches length were brought to the laboratory and made into a bundle. This was kept in a beaker containing 100 ml of 0·1% aqueous colchicine, which covered the lower half of the cuttings. After two hours, the twigs were taken out, washed well in running water and planted in the field to sprout. Out of 25 cuttings, only 9 plants survived to maturity.

Normal peg and pod formation were noticed in one plant (Fig. 1), which showed 38·7% pollen fertility as against 17·5% in the untreated triploid. It had a pollen diameter of 52·01 μ, while the triploid pollen grains measured only 40·40 μ. There was no gigasness in the morphology of the plant, but it was spreading and flowering profusely.



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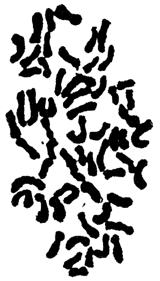

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FIGS. 1–3. Fig. 1. Twigs from sterile triploid (right) and colchicine induced fertile amphidiploid plants (left) to show normal pod setting. Fig. 2. Mitotic metaphase in triploid ($2n = 30$) (× 1,000) with camera lucida drawing. Fig. 3. Mitotic metaphase in amphidiploid ($2n = 60$) (× 1000) with camera lucida drawing.

The amphidiploid nature of this plant was confirmed by counting the somatic chromosomes ($2n = 60$), from fresh squashes prepared from side roots of the live plant (Fig. 3). The triploid showed $2n = 30$ chromosomes (Fig. 2). However, meiosis of this amphidiploid plant could not be studied due to unforeseen reasons. A total of 30 seeds have been collected from this plant. The amphidiploid plant has been successfully propagated by stem cuttings both for meiotic analysis and for utilization as bridging species in further backcrosses to cultivated groundnut.

A fertile allohexaploid with $2n = 60$ chromosomes was obtained by Kumar *et al.*[2], by treatment with colchicine (0·2%) of young vegetative buds of the hybrid between *A. hypogaea* × *A. villosa.* Further, D'Cruz and Upadhyaya[1] gave cytological evidence to show that *A. hypogaea* is a typical allotetraploid, based on the behaviour of the interspecific hybrid between *A. hypogaea* × *A. villosa*, and their C_1 and C_2 progenies. Information on the utilization of the synthetic amphidiploids as bridging species, between cultivated and wild species, for transfer of tikka resistant genes is lacking. In the present study the wild species used (*A. duranensis*), the interspecific hybrid and the allohexaploid plant were found to be tolerant to *Cercospora* leaf spot (tikka) disease. Further work on the cytology of the vegetatively propagated amphidiploids and their back cross derivatives with the cultivated species is in progress.

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---

1. D'Cruz, and Upadyaya, B. R., *Ind. Oil Seeds Jour.*, 1962, 33 (1), 33.
2. Kumar, L. S. S., D'Cruz, and Oke, J. G., *Curr. Sci.*, 1957, 26, 121.
3. Seetharam, A., Nayar, K. M. D., Sreekantaradhya, R. and Achar, D. K. T., *Cytologia*, 1973, 38, 277.
4. —, —, Achar, D. K. T. and Hanumanthappa, H. S., *Curr. Res.*, 1974, 3, 98.

A NEW SPECIES OF *SPIROTRICHONYMPHA* GRASSI AND FOA (PROTOZOA: MASTIGOPHORA) FROM A XYLOPHAGOUS TERMITE FROM INDIA

WHILE examining the termite flagellates from Hooghly district, West Bengal, the author discovered a new flagellate from *Coptotermes heimi* (Wasm.) which is described here as *Spirotrichonympha bhadreshwarensis* sp. nov.

The specimens obtained from the gut contents of the host were examined in fresh condition, fixed in Schaudinn's fluid and stained with Heidenhain's iron haematoxylin. All the measurements were taken with the aid of a calibrated ocular micrometer and drawings were made with the help of camera lucida.

Description.—The body is more or less pear-shaped (Fig. 1) with pointed anterior end and slightly compressed posterior end, 22·5 to 67·5 μ in length (average

50·4 μ) and 18 to 34·5 μ in breadth (average 27·3 μ). A very short tubule originates at the anterior end and does not reach to the nucleus. The sprial bands are dexiotropic. The number of spiral bands of flagella vary from 19 to 22 in adult and 10 to 15 in oval bodied young forms. A little of the posterior end of the body is devoid of spiral bands in both adult and young forms. The anterior flagella around the tube measure 7·5 to 19·5 μ in length and the posterior ones 10·5 to 30 μ in length. The spherical nucleus which measures 4·5 to 7·5 μ in diameter has scattered chromatin granules and is located in the anterior third of the body. Six chromosomes in paired condition have been observed at the periphery attached to nuclear membrane. Various stages of nuclear division have, however, not been encountered. The axostyle was not found in the new species dealt with and in this aspect agrees with the observations of Koidzumi, (1921)[1] and Chakravarty and Banerjee (1956)[5]. Mackinnon, (1927)[1]; de Mello, (1921)[3] and Karandikar and Vittal (1954)[4], however, described axostyle in other species. Endoplasm contains fragments of wood particles of various sizes.

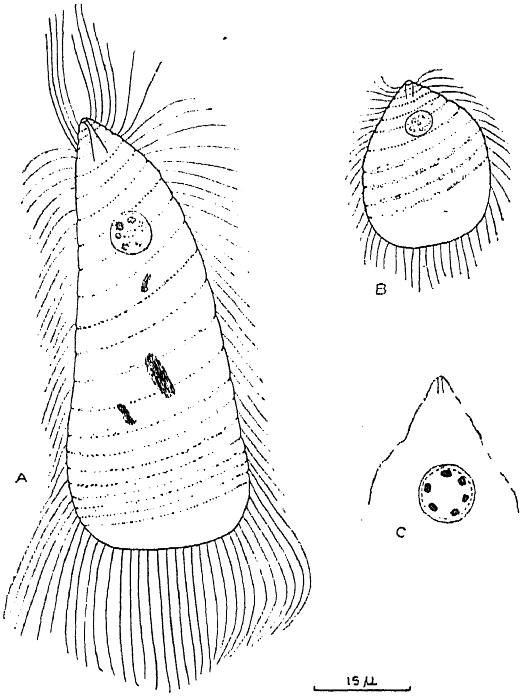

FIG. 1. *Spirotrichonympha bhadreshwarensis* sp. nov. A, adult flagellate; B, young form; C, anterior part of the flagellate showing six chromosomes in paired condition.

Remarks.—So far eleven species of *Spirotrichouympha* have been described. The present species resembles to *S. pyriformes* Chakravarty and Banerjee in the shape of body, absence of axostyle and in the shape of nucleus but differs in the body length 22·5 to 67·5 μ *versus* 10·70 to 55·64 μ and breadth 18 to 34·5 μ *versus* 8·56 to 21·40 μ. The anterior and the posterior flagella are larger than *S. pyriformes.* Six chromosomes have been observed in the present species as against five in *S. pyriformes.* The present species also resembles *S. leidyi* Koidzumi in having similar shape but the latter can conveniently be separated due to the length ranging from 22·5 to 67·5 μ *versus* 15 to 50 μ and breadth ranging from 18 to 34·5 μ *versus* 8 to 30 μ. Further, the nucleus in *S. leidyi* is oval and located in the middle region of the body whereas in the present species it is spherical and situated in anterior third. The present species also shows affinities to *S. elegans* Mackinnon in body shape and structure of the tubule but can be distinguished from *S. elegans* in the number of spiral bands 22 *versus* 16. The number of chromosomes are six instead of four as in *S. elegzns.* From the above conjecture, the present form is described as *Spirotrichonympha bhadreshwarensis* sp. nov. The species name is given here after the type locality.

Type host : *Coptotermes heimi* (Wasm.)

Type locality: Bhandreshwar, Hooghly district, West Bengal

Holotype : On the slide wil be deposited in Zoological Survey of India.

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---

1. Koidzumi, M., *Parasitology,* 1921, 13, 235.
2. Mackinnon, D. L., *Quart. J. micr. Sci.*, 1927, 71, 47.
3. de Mello, F., *Arquit. Indo-portug. Med. Hist. Nat.*, 1921, 1, 177.
4. Karandikar, K. R. and Vittal, M., *Jour. Univ. Bombay,* 1954, 23, 1.
5. Chakravarty, M. M. and Banerjee, A. K., *Proc. Zool. Soc., Bengal,* 1956, 9, 35.

XENIA IN INTERSPECTIFIC CROSSES OF SORGHUM

XENIA may result in crosses between lines that differ for a single dominant oligo-gene affecting endosperm characters like aleurone colour in maize[3]. Paternal effects are often met with, in diverse crosses where the effects of the paternal genes are dominant over the maternal effects as can be seen in crossed seed (increase in size of the fruit and

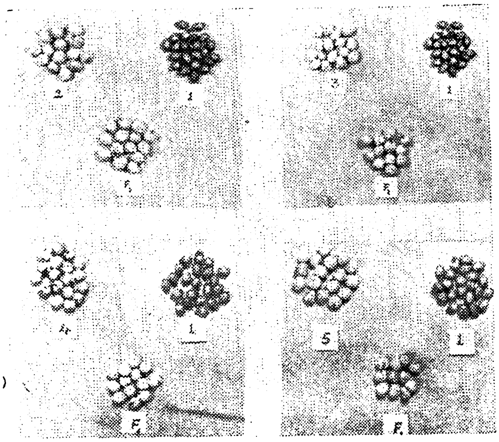

FIG. 1. Showing the appearance of the F_1 seeds. 1. *S. sudanense*; 2. Swarna; 3. *S. caffrorum* 4. 14–8–81–1–1; 5. 148.

embryo of the seed and loosening of wrinkles in sweet corn, pollinated with other sub-species[1] and seeds with teosinte characteristics when flint maize was pollinated with teosinte pollen[2]). In the present study paternal effects are recorded for seed size and shape, embryo size and shape, and general appearance of the grain.

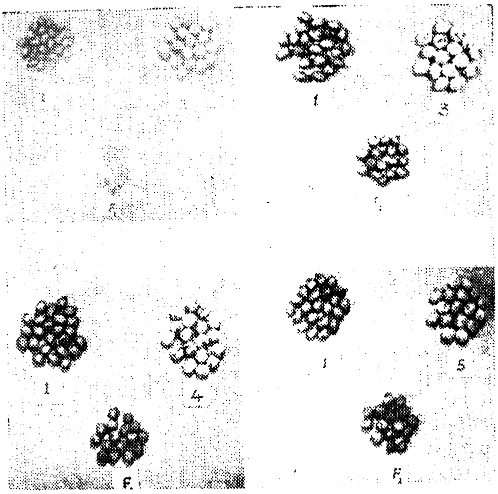

FIG. 2. Showing the appearance of the F_1 seeds in reciprocal crosses.

Seed characteristics of five parents and their eight crosses are given in Table I along with paternal effects indicated therein for each cross. Photographs are presented to show the general appearance of the grains. The shape of the grains in all the four crosses with the common female parent,

TABLE I

Paternal and maternal effects for seed characteristics in Sorghum Crosses

	Seed size	Seed shape	Embryo size	Embryo shape	General appearance	Seed colour
Parents:						
S. sudanense	Small	Ovoid	Small	Elliptical		Brown
S. caffrorum	Medium	Eliptical	Small	Ovoid		White
S. bicolor Swarna	Bold	Round	Large	Ovoid		White
14–8–81–1–1	Small	Round	Small	Ovoid		„
148	Medium	Round	Large	Ovoid		„
Crosses:						
S. sudanense × Swarna	Small	Round*	Large*	Oviod*	*	Brown
„ × 148	Medium*	Round*	Large*	Ovoid*	*	„
„ × 14–8–81–1–1	Small	Round*	Small	Elliptical	*	„
„ × *S. caffrorum*	Small	Round*	Small	Elliptical	*	„
Reciprocal crosses						
Swarna × *S. sudanerse*	Similar to corresponding female parents					White
148 × „						„
14–8–81–1–1 × „						„
S. caffrorum × „						„

i.e., S. sudanense, is changed like the paternal parent (Fig. 1).

Xenia was expressed only when *S. sudanense* was used as a female parent. From the observations (Fig. 2), it is clear that the seed characteristics of *S. bicolor* and *S. caffrorum* are dominant over those of *S. sudanense*. Xenia is expressed because of simple dominance of the male parent for the seed characters. But, Xenia was not expressed for all the characteristics in every cross. The only cross in which xenia was expressed for all the characteristics studied was *S. sudanense* × 148. However seed colour appears to have maternal effect.

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---

1. Kozubenko, V. E., *Breeding for Improved Chemical Composition,* Kukuruza (maize), 1965, No. 11, 29.
2. Rasidov, J. R., *Uzbek biol.*, 1968, No. 2, 78.
3. White, O. E., *Amer. Jour. Botany*, 1917, 4, 396.

ROLE OF pH IN THE FRUIT ROT OF CHILLIES CAUSED BY *COLLETOTRICHUM CAPSICI*

THE pectic enzymes produced by fungal pathogens have sharp peaks of activity at certain pH values, so that the initial pH of a tissue and the change in pH caused by the pathogen may be significant[1]. The significance of the rise in pH during disease development has been emphasised by several workers. Hancock[2] suggested that processes that induce changes in pH during pathogenesis could contribute significantly to disease development if the activities of certain degradative enzymes were favourable.

TABLE I

Effect of pH on the time for maceration of green and red chilli fruit discs by diseased tissue extract

Fruit tissue discs	pH of the diseased tissue extract						
	3·5	4·5	5·5	6·5	7·5	8·5	9·0 and above
Green	(No maceration)			12 h	8 h	6 h	5 h
Red	No maceration	24 h	8 h	7 h	5½ h	4 h	3½ h

The fungus *Colletotrichum capsici* causing fruit-rot disease in chillies (*Capsicum annuum* L.) produces an actively macerating endo-polygalacturonate *trans*-eliminase (PGTE) *in vivo*[3]. Its optimum activity was near pH 9·1. Other pectic enzymes could not be detected.

Extracts obtained from the diseased tissue were tested for their macerating activity on chilli fruit discs over a pH-range of 3·5–9·5. Time taken for the loss of coherence of cells was noticed. Desired pH values were maintained by using the 3 buffers suggested by Tribe[4] for maintaining uniform concentration of the buffering salts.

As shown in Table I the maceration was rapid at pH 9·0 and above. At any pH, red chilli discs were more readily macerated than the green ones. Data at pH 5·5 are critical since these are the approximate pH values of healthy fruits. At pH 5·5 green chilli discs were not macerated while the red ones were. This is what is perhaps happening *in vivo* when the fruits are infected by the fungus. In nature, the disease due to *C. capsici* is common only in red ripe chillies. In the case of green chilli fruits maceration will not be brought about when they are attacked by the fungus. On the other hand, the red ones are macerated by the enzyme produced by the invading fungus.

A change in pH favours the predominating enzyme action[2]. Since the pH of the infected chilli tissue rose to 8·3 during lesion development, it would appear that only the *trans*-eliminative enzymes were operative in bringing about the rotting. Similar report was made in the case of stem rot of squashes[5]. Thus pH appears to be a key factor to explain why green chillies do not get the *Colletotrichum*-rot while the red ripe ones get.

The initial pH of the chilli fruits, which is around 5·0, would be favourable only for exo- or endo-polygalacturonase (PG) activity, if there was any. *C. capsici* produced moderately active exo- and endo-PG *in vitro*, but these were not detectable in the infected fruits.

It should however be mentioned here that in some instances the green fruits turn red at the site of infection apparently due to the ripening processes setting in locally. In such cases the green fruits also become susceptible and the disease spreads progressively but slowly.

A (non-dialyzable and thermostable) 'Killing factor' is also produced by the fungus *C. capsici* in culture[6]. Its activity is rapid below pH 5·5. This non-enzymic factor may also be playing some role in rapidly killing the host tissue especially before the pH drifts to alkalinity. In other words, the 'Killing factor might be playing a role in the initial stages (pH is around 5·0) while the endo-PGTE could assume importance both at the initial stages and to an increased extent at subsequent invasion by the fungus (when the pH increases to 8·5).

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---

1. Wood, R. K. S., *Plant Pathology, An Advanced Treatise*, 1960, 2, 233.
2. Hancock, J. G., *Phytopathology*, 1966, 56, 975.
3. Thirupathaiah, V., *Phytopath. Z.*, 1972, 75, 175.
4. Tribe, H., *Ann. Bot.*, 1955, 19, 351.
5. Hancock, J. G., *Phytopathology*, 1968, 58, 62.
6. Thirupathaiah, V., *J. Indian Bot. Soc.*, 1974, 53, 261.

PHENOLIC ACIDS OF VIABLE AND NON-VIABLE SEEDS OF GROUNDNUT (*ARACHIS HYPOGAEA*, L.)

THE various factors involving the loss of seed viability were discussed by Roberts[1] and various physiological and biochemical changes associated with seed deterioration were reviewed by Abdul Baki and Anderson[2]. Dey *et al.*[3] reported that there was an accumulation of certain phenolic acids like coumarin, ferulic and sinapic acids in the non-viable seeds of rice. The present paper reports investigation on qualitative and quantitative aspects of phenolic acids in viable and non-viable goundnut seeds.

Four months old (viable) and three year old (non-viable) seeds of groundnut variety TMV-3 were used for qualitative and quantitative determination of individual phenolic acids. The viability of seeds was tested with tetrazolium chloride and by the normal germination test.

Phenolic acids were extracted from both viable and non-viable seeds by the method of Bate-Smith[4]. Two dimensional paper chromatography was carried out to detect the individual phenolic acids as per the technique of Ibrahim and Towers[5]. The individual phenolic acids from the paper were eluted with 95% ethanol and co-chromatographed with authentic samples. The phenolic acid content was estimated with Folin's reagent.

Six monohydroxy phenolic acids (*α*-resorcylic, *p*-hydroxybenzoic, *trans-p*-coumaric, *cis-p*-coumaric, phloretic and vanillic acids) and two dihydroxy phenolic acids (protocatechuic and chlorogenic acids) were present in the viable seeds. In non-viable seeds the number of monohydroxy and dihydroxy phenolic acids remains the same as viable seeds except protocatechic acid. However, more amounts of monohydroxy phenolic acids were present in the viable seeds when compared to non-viable seeds. The content of dihydroxy phenolic acids decreased to half of their amounts in the non-viable seeds. Thus, the total disappearance of photocatechuic acid and decreased levels of all the individual phenolic acids in the non-viable seeds may be suggestive of their primary role similar to essential metabolite levels like carbohydrates, fats and proteins which were shown to decrease in non-viable seeds when compared to viable seeds[1]. It has been shown that large amounts of inhibitors like abscisic acid accumulate in non-viable seeds of groundnut[6]. Therefore, it is quite probable that the phenolic inhibitors do not play an active role in the loss of viability of groundnut seeds.

TABLE I

Phenolic acids of viable and non-viable groundnut seeds μg/gm dry seed

	viable	non-viable
1. Chlorogenic acid	44·55	20·20
2. Protocatechuc acid	9·27	..
3. *Trans-p*-coumaric acid	7·69	2·36
4. *p*-Hydroxy benzoic acid	12·15	3·23
5. *α*-Resorcylic acid	16·20	4·01
6. *cis-p*-coumaric acid	10·07	4·81
7. Phloretic acid	6·68	1·80
8. Vanillic acid	5·47	4·61
Total	110·08	41·02

The authors thank Professor V. S. Rama Das, for providing facilities.

Dept. of Botany, S. BALANARASIMHA REDDY.
Sri Venkateswara K. N. RAO.
University, P. M. SWAMY.
Tirupati 517 502 (A.P.), *December* 8, 1976.

---

1. Roberts, E. H., In: *Viability of Seeds*, Ed. E. H. Roberts, Chapman and Hall, Ltd., London, 1972.
2. Abdul Baki, A. A. and Anderson, J. D., *Seed Biology*, 1972, 2, 283.
3. Dey, B., Sircar, P. K. and Sircar, S. M., *Proc. Int. Symp. Pl. Growth Substances*, Calcutta, 1967.
4. Bate Smith, E. C., *Biochem. J.*, 1954, 58, 122.
5. Ibrahim, R. K. and Towers, C. H. N., *Arch. Biochem. Biophys.*, 1960, 87, 125.
6. Narasimha Reddy, S. B. and Swamy, P. M., *J. Exp. Bot.*, 1976 (In press).

CONTROL OF 'CHENTHAL' (BACTERIAL BLIGHT) DISEASE OF CARDAMOM WITH PENICILLIN

CHENTHAL disease of Cardamom caused by *Corynebacterium* sp. was the first bacterial disease reported by Mathew George *et al.*[1] on the host *Eletteria cardamomum* (L.) Maton. This is a destructive disease causing severe crop losses in the high ranges of Kerala. Six antibacterial compounds were screened in an attempt to control the disease. Penicillin was found most effective in experimental field trials.

For comparative evaluation, sensitivity discs (Bharath Laboratories, India) were placed in nutrient agar plates seeded with the pathogen. After 24 hr of growth the inhibition zone was measured. Penicillin was found most effective. Kanamycin, Sulphadiazine, Erythromycin and Tetracycline inhibited growth partially, while Streptomycin was ineffective. In bioassay, using tube dilution technique, penicillin at 1 mcg/ml concentration completely inhibited the growth of the pathogen.

Field trials were undertaken at Kalarikal Estate, Vadanmedu, Kerala. Twenty-five clumps from a diseased spot were selected, each having an average of 15 to 20 pseudostems. The plants were sprayed with 100 mcg/ml solution of penicillin for three consecutive days followed by a second round of spray after a lapse of 30 days. Out of the 25 clumps, 10 were selected at random and 3 pseudostems from each were taken for disease observations. Water-soaked lesions, the first visible symptoms (Mathew George *et. al.*[1]) on the youngest three leaves were counted. Observations were recorded at 30 and 60 days after the initial spray. The average number of lesions in relation to number of leaves of comparable age has been consolidated and presented in Table I. The increase in the number of lesions recorded after 30 days of treatment is attributed to the rainy weather which is congenial to the pathogen. Results of the second observations revealed the efficacy of penicillin as a proper control against the disease. Improvement of the treated plants was marked, while the untreated controls deteriorated.

Under field conditions 217 clumps in another plot of experimental plants were similarly sprayed with penicillin. The plants showed improvement after 60 days. The feasibility of using penicillin because it is rapidly translocated has been indicated elsewhere[2].

TABLE I

	Average number of lesions per leaf		
	I leaf	II leaf	III leaf
Pre-treatment observation	2·6	26	40·3

	Treated			Control		
	I leaf	II leaf	III leaf	I leaf	II leaf	III leaf
I observation	11·3	57·5	45·3	25	78·1	51·4
II observation	1·11	14·3	37·4	44·4	93·3	90·2

We thank Mr. Sajan Kurian of Kalarikal Estate who brought the disease to our notice and Dr. J. V. Bhat, Professor Emeritus, for inspiration.

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December 3, 1976.

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1. Mathew George, Thomas Joseph, Potty, V. P. and Jayasankar, N. P., *J. Plant. Crops*, 1976, 4, 23.
2. David Pramer, In: *Advances in Applied Microbiology*, Ed. Umbreit, W. W., Academic Press, London, 1959, 1, 78.

DISEASES OF FRUITS FROM HARYANA
A NEW LEAF SPOT DISEASE OF *ZIZYPHUS MAURITIANA* LAMK.

DURING October-November, 1975, a new leaf spot disease of Ber (*Zizyphus mauritiana* Lamk.) var. *Sandhura–Narnaul* and *Rashmi* was observed in the orchard of Haryana Agricultural University. The disease is characterised by foliar sooty tuft like circular to irregular black spot. When infection advances, it covers a large area on the lower surface of the leaves and upper surface shows brownish discoloration (Fig. 1).

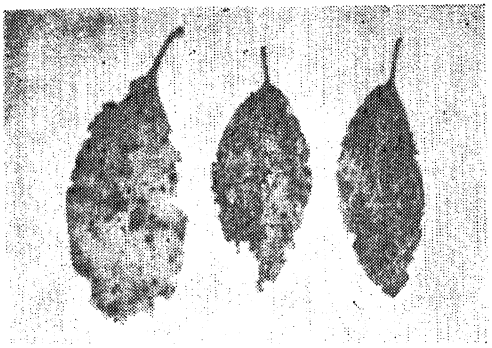

FIG. 1. The leaves of Ber showing typical symptoms caused by *Isariopsis indica* var. *zizyphi*.

The fungus was isolated on Czapek's agar medium and pathogenicity was proved on fresh and healthy leaves. Morphology of the fungus, resembles with *Isariopsis indica* with marked differences in size of conidia and synnemata[1] (Fig. 2). Therefore, a new variety, *viz.*, *Isariopsis indica* var. *zizyphi* is proposed to accommodate it.

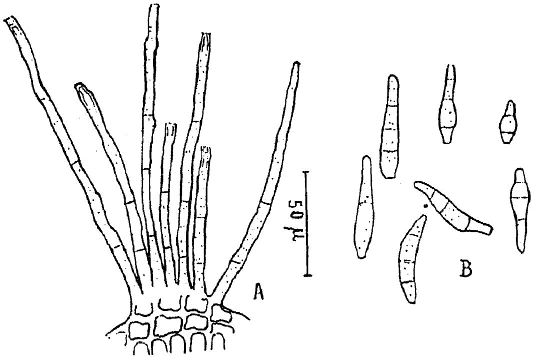

FIG. 2. A. Synnematum, B. Conidia (camera lucida).

Isariopsis indica var. *zizyphi* var. nov.

Infection on lower surface of the leaves in the form of black colour, circular to irregular spots measuring 0·5–5 mm. Synnemata dark olivaceous, divergent, measuring 220 × 102 µ (average) composed of loose conidiophores. Conidiophores. erect, simple, olivaceous in colour, bears conidia terminally and laterally, measuring 54·4–138·4 × 6·8 µ. Conidia olivaceous, 0–3 septate, cyclindrical to obclavate, sometimes pyriforms measuring 17·0–44·2 × 8·5–10·2 µ.

Isariopsis indica var. *zizyphi* var. nov.

Infections maculae foliicolae, hypogenae, nigris, circulares vel irregulares, 0·5–5 mm diameter. Synnemata fusce olivacea, divergentia, 220 × 102 µ. mediet. Conidiophores recti, simplices, olivaceis, supportantes. Conidia singula terminaliter et lateraliter 54·4–138·4 × 6·8 µ. Conidia olivaceis 0–3 septata, cylindrica vel obclavatis, raro pyriformibus, 17·0–44·2 × 8·5–10·2 µ.

The type specimen is deposited in Herb. Plant Pathology Laboratory, HAU, Hissar (PPHAU 70), and Herb. Crypt. Ind. Orient, IARI, New Delhi.

Thanks are due to Head of the Department of Horticulture, HAU, Hissar, for providing the laboratory facilities.

Department of Horticulture, P. C. GUPTA.
Haryana Agril. University, R. L. MADAAN.
Hissar, *November* 2, 1976.

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1. Nair, G., *Curr. Sci.*, 1964, 33, 25.

REPRODUCTIVE BIOLOGY OF *BIDENS PILOSA* L. (COMPOSITAE)

THE following is a preliminary report on seed germination, mitosis, meiosis, pollen fertility and germination of pollen grains, seed-setting and flavonoid chemistry of 15 populations of South Indian materials of this aggressive weed carried out at the Jodrell Laboratory, Kew, U.K.

(1) Fresh seeds gave 100% germination within 36 hours of sowing, with a peak of germination between 27° C and 31° C. (2) Root tips pretreated with alpha-bromonaphthalene for four hours at 18° C gave clear counts of $2n = 72$; an occasional satellite was seen (Fig. 1). (3) Meiotic preparations (36 bivalents)

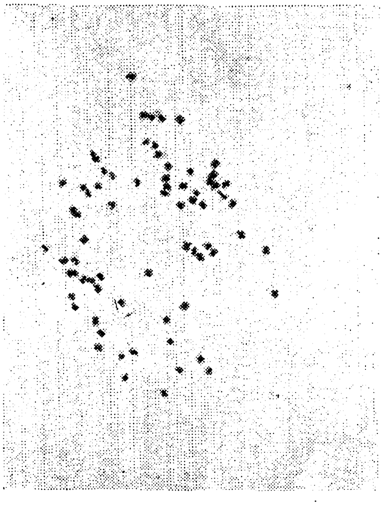

FIG. 1. *Bidens pilosa* L. Mitosis showing $2n = 72$; one satellite visible.

showed single or double chromosome bridges (Fig. 2). (4) Pollen grains (*c.* 225 per anther) gave cent per cent germination and the growth of the pollen tube was normal (observed under uv microscope). (5) Copious seed-setting occurred even when selfed (by early bagging of capitula) and the resulting seeds germinated as fast as seeds from crossing. (6) Caffeic acid, aurones and chalcones were the main flavonoids from leaf extracts.

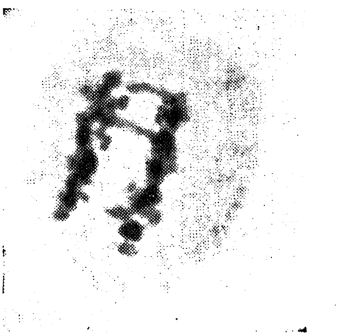

FIG. 2. *Bidens pilosa* L. Meiosis, showing a double chromosome bridge.

The Rapinat Herbarium,
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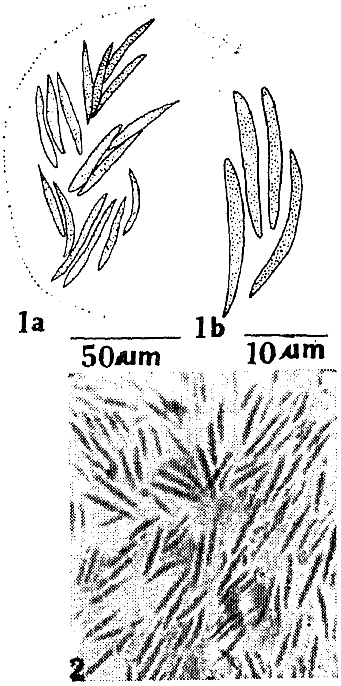
GENUS *QUADRIGULA* PRINTZ.—A NEW ADDITION TO THE INDIAN FLORA

THE genus *Quadrigula* Printz. belongs to order chlorococcales, which is well represented in the Indian flora and of which 53 genera and 208 species are known to occur in this country[1]. Depending on taxonomic opinion, the order comprises a total of 173–180 genera and 900–1,079 species, distributed over 10 to 14 families[1-3]. The genus *Quadrigula* is placed in the Selenastraceae or the Oocystaceae[1-5]. The Selenastraceae includes 14 genera out of which only 6 are known from India[1]. *Quadrigula* has not been recorded from this country so far. The present communication records the occurrence of this genus in the Indian flora and briefly describes the Indian alga.

The alga was collected from a paddy field near Lucknow, U.P., during the course of an investigation on the algal flora of Crop-fields of Uttar Pradesh. It was also isolated from soil-water enrichment cultures of the soil of the same paddy field in the month of September 1974. Both in nature and in culture, the alga forms a thin floating scum on the surface made up of groups of cells embedded in a gelatinous envelope (Fig. 1 *a*).

The cells are generally in groups of two, three or four, although solitary cells are not uncommon. The individual cells are fusiform, elongated and straight with inner margins slightly concave. The cells are arranged with their longitudinal axes parallel and are 2·0–5·0 μm in breadth and 15·0–30·0 μm in length. Each cell possesses a single chloroplast filling almost the entire cell; pyrenoids are absent (Figs. 1 *b* and 2).



FIGS. 1–2. *Quadrigula lacustris.* Fig. 1 *a.* Groups of cells embedded in a gelatinous envelope. Fig. 1 *b.* A group of four cells. Fig. 2. Cells arranged in groups of 2-4 (× 300).

The present alga agrees well with the available descriptions of *Q. lacustris* (Chodat) G. M. Smith, but has slightly longer cells and lacks pyrenoids. It is, therefore, identified as that species.

One of the authors (RKM) gratefully acknowledges the financial assistance received from the University Grants Commission, New Delhi, during the tenure of which this work was undertaken.

Department of Botany,
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Lucknow 226 007, *October* 28, 1976.
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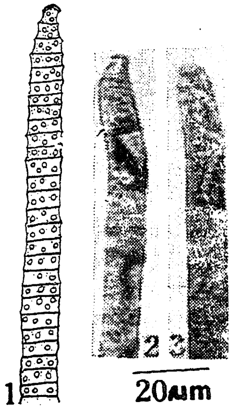
1. Philipose, M. T., *Chlorococcales*, I.C.A.R., New Delhi, 1967, pp. 365.
2. Bourrelly, P., *Les Algues D'eau Douce. I. Algues vertes.* Boubee et Cie, Paris 1972, pp. 572.
3. Fritsch, F. E., *Structure and Reproduction of the Algae*, Cambridge, 1935, **1**, 799.
4. Prescott, G. W., *Algae of the Western Great Lakes Area*, WM. C. Brown, Iowa, 1970, pp. 977.
5. Smith, G. M., *The Fresh Water Algae of the United States*, McGraw Hill and Co., New York, 1950, pp. vii + 718.

ON A MORPHOLOGICALLY INTERESTING *OSCILLATORIA* Sp.

In the course of investigations on the algal flora of crop-fields of Uttar Pradesh, an *Oscillatoria* sp. was isolated from enrichment cultures of soil from a potato (*Solanum tuberosum*) field near Lucknow, which shows a very interesting morphological feature hitherto unrecorded in the genus. This is also the first report of any algal taxon from soils of a potato field. The present taxon is briefly described as follows:



FIGS. 1–3. *Oscillatoria* sp. showing the serrate, nature of the margins.

Trichomes single or in groups, intermingled with other algae, olive to blue green, straight, ends slightly bent and attenuated; cells 12·0–14·0 μm broad, 4·0–6·0 μm long; granulated, 2–4 granules in each cell; septa projecting over the general plane of the cell wall in the shape of an annular rim, producing a serrate appearance; end-cell truncated, slightly bent, capitate with outer membrane thickened.

The present *Oscillatoria* sp. can be compared in its cell dimensions with a number of species of the genus particularly with *O. limosa* Ag. ex. Gom. and *O. sancta* (Kütz.) Gom.[1–4] but the present alga possesses an entirely different and remarkable feature in the presence of annulated septa, the margins of which protrude out slightly beyond the longtiudinal sides of the filament. These project out, giving the appearance of a more or less serrate margin (Figs. 1–3), whereas in other cases known so far in the genus, the trichomes may be constricted or unconstricted at the septa. The present plant is not assigned any specific taxonomic rank at present and only the unusual morphological observation is being recorded. Further culture work may, perhaps, elucidate the systematic status of the alga.

The authors gratefully acknowledgde the critical comments and suggestions from Dr. B. N. Prasad. The authors also acknowledge the financial assistance from the U.G.C. and the C.S.I.R., New Delhi, during the tenure of this work.

Department of Botany,
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November 4, 1976.

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---

1. Desikachary, T. V., *Cyanophyta*, ICAR, New Delhi, 1959, p. 686.
2. Geitler, L., *Cyanophyceae in Rabenhörst's Kryptogamen Flora*, Leipzig, 1932, p. 1196.
3. Prescott, G. W., *Algae of the Western Great Lakes Area*, W. M. C. Brown, Iowa, 1970, p. 977.
4. Tilden, J. E., *Myxophyceae in Minnesota Algae - I*, Minneapolis, 1910.

INHIBITION POTENTIAL OF AUREOFUNGIN AGAINST *CEPHALOSPORIUM SACCHARI* AN INCITANT OF SUGARCANE WILT.

Cephalosporium sacchari Butl. is an important pathogen causing sugarcane wilt. Aureofungin is a broad spectrum systemic antibiotic. It is found effective against a wide variety of fungi (Hindustan Antibiotics, Ltd., Leaflet). Aureofungin has been found useful in controlling several diseases under field conditions but no trial on sugarcane wilt has so far been made. The present communication is aimed to investigate the *in vitro* effect of Aureofungin on spore germination and mycelial growth of the pathogen.

Spore suspension was made in distilled water from a 10 days old culture of *C. sacchari* and centrifuged to render it free from mycelium and nutrients. For studying spore germination of the fungus, the spores were taken in a hanging drop culture using liquid Richards's medium. Aureofungin was added to liquid medium to give concentrations of 1, 5, 10, 20, 30, 50 ppm of Aureofungin keeping the control without the antibiotic. The hanging drop cultures, thus prepared, were placed in a moist chamber and incubated at 30° C for 48 hours. The percentage germination and the length of germ tubes were determined for each antibiotic concentration.

The *in vitro* effect of Aureofungin on mycelial growth of the fungus was made by "Poisoned food technique"[1]. Aureofungin was added to Richards's agar medium to give final concentrations of 5, 10, 20, 50, 100 ppm respectively with proper controls. Different concentrations of the medium were plated, inoculated with the disc of mycelium (5 mm diam.) punched out from vigorously growing cultures of the fungus and incubated at 30° C for 12 days. After incubation the growth was measured.

Results show that Aureofungin is inhibitory to spore germination of the pathogen at the minimal concentration used. The percentage germination of spores was 32 and the length of the germ tube was 53 μ at 1 ppm which further reduced with increase in concentration of the antibiotic. At 10 ppm and over germination percentage was nil.

The mycelial growth at 5 ppm of the antibiotic was 9 mm as compared to 64 mm in the control. The growth was completely checked at over 20 ppm.

The above findings confirm that Aureofungin has a potential utility against *C. sacchari* by virtue of its inhibitory effect on spore germination and growth of the pathogen even at the minimum concentration and the same can perhaps be useful in controlling the disease under field conditions.

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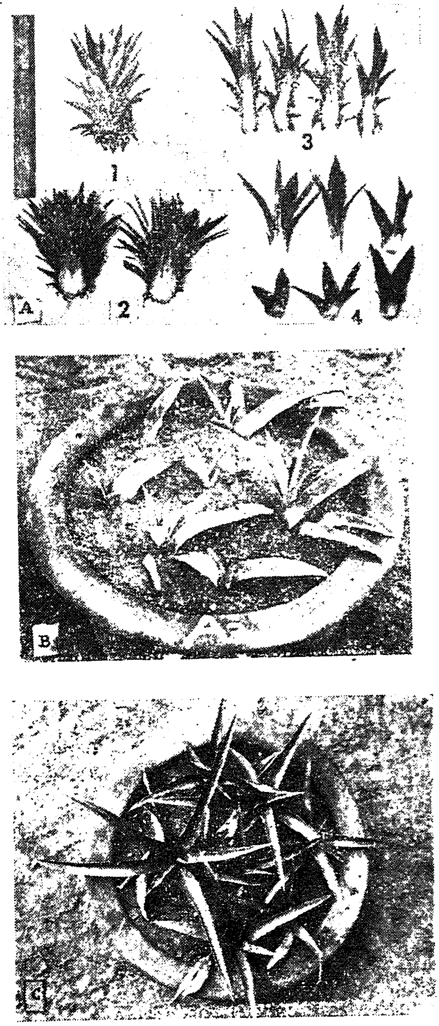
1. Nene, Y. L., *Fungicides in Plant Disease Control*, Oxford and IBH Pub. Co., New Delhi-1, 1971, p. 385.

VEGETATIVE MULTIPLICATION BY LEAF CUTTINGS OF CROWNS IN PINEAPPLE (*ANANAS COMOSUS* L.)

SHORTAGE of planting material is often felt in the case of pineapple. Reports of successful propagation using stumps, crowns, etc., are available. However, these methods are far from satisfactory for practical applications[1,2,3]. Hence efforts were made to use leaf cuttings (leaf with an axillary bud) from fruit crown of *Ananas comosus* L. Each leaf in the crown has a bud in its axil. Usually, crowns of fruits are discarded in India as they take more time for fruiting. Two experiments were laid, one in dry season (16th March 1976) and another in wet season (24th July 1976). Ten to sixteen longitudinal leaf cuttings were obtained from each crown. The crown was cut into 4 equal parts and then from each quarter piece, leaf cuttings were made (Fig. A). The first experiment in dry season was conducted in shade and open. Success upto 70% was obtained under shade in untreated leaf cuttings. Treatments with various fungicides like Bavistin, TBZ (thiobendazole), etc., did not increase the percentage success over untreated control. The second experiment in the monsoon season was done only in the open. The fungicides were applied just before planting by dipping the leaf cuttings for about five minutes in each fungicidal suspension. Success upto 100% was obtained with Dithane Z–78 (0·2%) compared to 62% in the case of untreated control. (Fig. B leaf cuttings, 3 months after planting and Fig. C leaf cuttings, 6 months after planting). This method can be used for solving the problem of shortage of plant material. Detailed results of the experiment will be reported elsewhere.



FIGS. A–C. Fig. A. Crown from fruit of pineapple (1), crown cut longitudinally into two pieces (2), each half piece further cut to make four pieces (3) and each quarter piece was cut to make leaf cuttings so that each cutting has 2–3 leaves. Fig. B. Success with propagation from leaf cuttings, three months after planting. Note small plants emerging from each leaf cutting. Fig. C. Growth of plants emerged from leaf cuttings, six months after planting.

The authors are thankful to Dr. G. S. Randhawa, Director, Indian Institute of Horticultural Research, for constant encouragement throughout the course of investigation.

Indian Institute of
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Bangalore-6,
November 30, 1976.

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H. S. SOHI.
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1. Evans, H. R., *Afr. Agr. J.*, 1952, 17, 179.
2. Macluskie, H., *Trop. Agriculture TRIN.*, 1939, 16, 192.
3. Seow, K. K. and Wee, Y. C., *Malaysian Agric. Jour.*, 1969, 47, 499.

DEVELOPMENT OF MULTIPLE RESILIFERS IN *LOPHA (ACTINOSTREON) DILUVIANA* (LINNÈ) FROM THE UPPER CRETACEOUS OF SOUTH INDIA

A SINGLE left valve of *Lopha* (*Actinostreon*) *diluviana* (Linné) showing multiple resilifers was collected from brownish calcareous sandstone of Trichinopoly group occurring at Anaipadi (11°, 06′ 78° 56′ 30″).

Though this species is fairly abundant both in the Utatur and the Trichinopoly groups at many localities[1], the phenomenon of the development of multiple resilifers is a rare one. Except this solitary individual all other specimens in our collection represent normal individuals with a single resilifer.

Ligamental area of this variant is abnormal in being very broad and nearly as tall as the rest of the valve height. Though the umbonal area and much of the posterior portion of the ligamental area is damaged, the undamaged portion bears at least five rèsilia which must have been functioning till the animal was living. Splitting of the resilifer areas is extremely irregular. One of the areas (*i.e.*, second from the anterior side) ends abruptly by joining midway with the adjacent area. All these areas have different widths.

A similarity between this specimen and a specimen of *Lopha semiarmata* (Böse)[2] from the Cardenas Formation (Maastrichtian) of Cardenas, San Lui Potosi State, Mexico, is that both these specimens have unusually thick shell walls and very tall ligamental area. The shell wall of the present South Indian specimen is nearly seven cm thick and has its ligamental area nearly six cm high.

Above commonness in characters of these abnormal individuals is very significant, as it further strengthens Lycett's view[3] that such variants are developed as a result of monstrosity. This explanation of Lycett is acceptable as unusually heavy shells formed due to monstrosity would need a sufficienetly strong ligamental area to operate effectively the opening and closing of the valves. Hence, such stray monstrous variants have very tall and broad ligamental areas with multiple resilia.

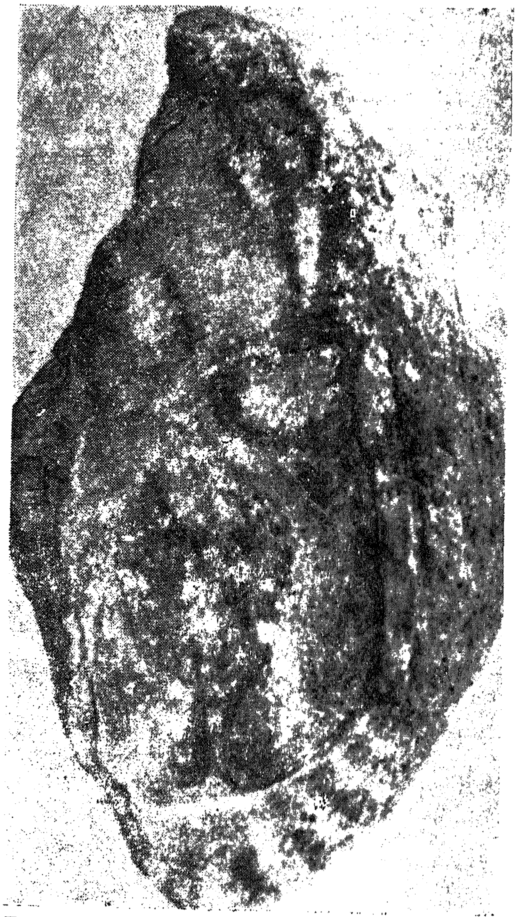

FIG. 1. LV of *Lopha* (*Actinostreon*) *diluviana* (Linné) showing multiple resilifers, × 0·80 (Sp. No. MACS G 666).

M.A.C.S. Research Institute,
Law College Road,
Poona 411 004, *November* 16, 1976.

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1. Stoliczka, F., *Pal. Ind.*, Ser. VI, 1871, Pt. 3, p. 9.
2. Böse, E., *Inst. Geol. Mexico, Bol.*, 1906, p. 24.
3. Stenzel, H. B., In : *Treatise on Invertebrate Paleontology*, Ed., R. C. Moore, 1971, Pt. N (3), Mollusca 6.

OCCURRENCE OF QUARTZ-MONZONITE PLUGS AT BORLAI-KORLAI IN KOLABA DISTRICT, MAHARASHTRA

THE Deccan Traps in Maharashtra, excepting in Bombay Islands, are considered to be made up of different types of basalts with intrusions of dolerite at number of places. In Bombay Islands, differentiates like trachytes, andesites and intrusives like diorites and lamprophyres have been reported (Sukheswala and Sethna, 1962)[1]. However, there is no record of occurrence of any such rock types in other parts of Maharashtra excepting one occurrence of lamprophyre reported by Deshpande and Chakranarayana (1974)[2] near Murud-Janjira along the west coast. On further investigation in the nearby areas the authors discovered two intrusive plugs of a rock, which on detailed examination were found to be quartz-monzonite.

These two plugs are exposed on the western coast of Maharashtra between villages of Korlai and Borlai near Salav on an extensively spread out beach (18° 10′ N, 72° 55′ E). They show an alignment in a roughly north-south direction and are separated from one another by a distance of about 1·5 Km.

The outcrops of both these plugs are approximately oval and their longer axes are along N 15° W–S 15° E direction. The northern of the two is barely above the high tide line while the southern one is exposed only during low tide. The northern body is about 50 m in length and 25 m in width. The southern body stands about 3 m high during low tide. The northern body forms a low mound of about 6 to 8 m height above the beach level. These two bodies consist of a medium to coarse grained mesocratic rock. Towards the centre of the plugs the rock becomes coarser.

Under the microscope the rock shows hypidiomorphic granular texture. It is composed of feldspar, granophyre, pyroxene, amphibole and biotite with epidote, apatite and opaques as accessory constituents. Plagioclase is 30–33% by volume while percentage of orthoclase also varies within the same range. The content of plagioclase is between 52% and 66%. The feldspars in the marginal portion show protoclastic structure (Harker 1964)[3] and saussuritisation. Granophyre varies from 13–24% by volume and shows an increase towards the margin at the expense of the feldspars. The pyroxene is mostly subcalcic augite (2V = 22°–30°) and varies between 5% and 12%. It is changed to hornblende (upto 6%), which in turn is changed to biotite (2%–6%), sometimes exhibiting corona structure. The marginal portions of the plugs show an increase in the proportion of biotite and hornblende. Quartz in this rock is of two generations; one belonging to granophyre and the other represented by euhedral to subhedral crystals occupying the interstices between the early formed crystals. Epidote and chlorite are present in small amounts in the marginal portions. On the basis of the mineralogical composition and the modal analysis, the rock is identified as quartz monzonite.

These two quartz-monzonitic plugs occur in a region which is dissected by quite a large number of dykes of varying mineralogical composition, some of which are coarse grained and are lamprophyric in composition. They form dyke swarms with varying trends. None of these dykes cuts through these plugs and therefore the dykes are older than the latter.

The two plugs of quartz-monzonite pose a puzzling problem as to what they represent. The occurrence of dyke swarms of varying composition, including the lamprophyres, is considered to be indicative of the sites of ancient volcanic centres (Turner and Verhoogen, 1962)[4]. Deshpande and Chakranarayana have suggested this possibility while reporting the occurrence of lamprophyres from Murud–Janjira. It may therefore be suggested, that these two plugs represent the deep seated portions of the conduits of such volcanoes in which by differentiation of the basaltic magma which was being poured out to form the lava flows, quartz-monzonite was formed. However, a possibility that these two plugs could be the upward extended appendages of a deeper seated pluton cannot be ruled out.

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1. Sukheswala, R. N. and Sethna, S. F., *Jour. Geol. Soc. India*, 1962, 3, 125.
2. Deshpande, G. G. and Chakranarayana, A. B., *Curr. Sci.*, 1973, 42 (11), 404.
3. Harker, Alfred, *Metamorphism*, Methuen and Co. Ltd., London, E. P. Dutton and Co., Inc., New York, 1964.
4. Turner, F. J. and Verhoogen, J., *Igneous and Metamorphic Petrology*, Allied Pacific Pvt. Ltd., Bombay, 1962.

SHORT SCIENTIFIC NOTES

Some Newer Cyanoethylated Cyanoacetanilides, Malonanilides and Ethyl Malonates as Possible Insecticides

Different cyanoethylated compounds have been shown to exhibit biological activity[1,2]. Twentytwo substituted α-cyano, α, α-*bis*-(β-cyanoethyl)- malondianilides, α, α-*bis*-(β-cyanoethyl) malonanilates and α, α-*bis*-(β-cyanoethyl) acetánilides have been prepared and were found to possess sufficient insecticidal activity. The activity of these compounds was compared with D.D.T., B.H.C. and pyrethrum.

The maximum insecticidal activity was observed with α, α-*bis*-(β-cyanoethyl) 3, 3'-dichloromalondianilide. It seems that the nucleophilic groups decreased the activity and electrophilic groups increased the insecticidal activity α, α-*bis*-(β-cyanoethyl) malonanilate derivatives were found to possess considerable activity. Maximum activity was observed with α, α-*bis*-(β-cyanoethyl)-malon-4-chloranilate (about 70% as compared to DDT or BHC).

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1. Shukla, J. S. and Bhatia, P., *J. Ind. Chem. Soc.*, 1976; 53, 19.
2. —, *et al.*, *Ibid.*, 1976, 53, 375.

Components of *Cestrum nocturnum*

Cestrum nocturnum Linn. (Solanaceae) (night queen) is grown as an ornamental plant all over India[1]. This was studied earlier for its chemical constituents[2,3]. The present work records for the first time the isolation of ursolic acid, β-sitosterol-β-D-glucoside and pseudoyuccagenin.

Shade-dried leaves were extracted with hot ethanol (95%) under reflux. The aqueous concentrate was repeatedly shaken with *n*-hexane, ether and ethyl acetate in succession. The hexane washings on column chromatography over neutral alumina yielded a low melting solid, m.p. 85° ; the fractions from the chloroform eluate gave colourless crystals, m.p. 280–81°, $[\alpha]_D + 68°$ (py), M.F. $C_{30}H_{48}O_3$. It gave colour reactions characteristic of a triterpenoid. On acetylation with Ac_2O and pyridine, it formed an acetate, m.p. 279–81° and a methyl ester, m.p. 168–69° on treatment with CH_2N_2. It was identified as ursolic acid by direct comparison with authentic samples of the compound and its derivatives by m.m.p. and co-TLC.

The ether-soluble fraction also yielded some more ursolic acid when chromatographed over silica gel. Elution with chloroform : methanol (9 : 1) yielded β-sitosterol-β-D-glucoside, m.p. 284–85° (a), giving all characteristic colour reactions ; it formed a tetra-acetate, m.p. 170° and gave β-sitosterol, m.p. 129–31° and D-glucose on acid hydrolysis. The identity of the glucoside was further confirmed by a direct comparison.

The mother liquor left after extraction was combined and mixed with ammonium hydroxide and kept overnight. Repeated purification of the precipitated solid gave a crystalline compound m.p. 192° (d). This was identified as pseudoyuccagenin by converting it to yuccagenin, m.p. 239–40° C, by heating it with 2% alcoholic HCl[2].

Ursolic acid was reported to be present in *Cestrum diurnum*[4]. It is now isolated in a better yield (0·4%) from a sister species, *C. nocturnum*.

We are thankful to the Principal and Prof. M. N. Ghosh of our Institute for encouragement.

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1. Chopra, R. N., Chopra, I. C. and Varma, B. S., *Supplement to Glossary of Indian Medicinal Plants,* Publications and Information Directorate, New Delhi, 1969, p. 15.
2. Chakravarti, R. N., Dutta, S. and Mitra, M. N., *Bull. Calcutta School Trop. Med.*, 1963, 11, 56.
3. —, Chakravarti, D., Dutta, S. and Mitra, M. N., *J. Sci. Ind. Res.*, 1969, 28, 26.
4. —, Dutta, S. and Mitra, M. N., *Experientia,* 1964, 20, 200.

Observations on the Leptophragma of *Chrysochroa* (Buprestidae)

Saini (1964) described the leptophragmata in cryptonephric beetles and mentioned that all cryptonephric beetles, except Anobiids, Ptinids and Melolontha, are leptophragmic. However, Buprestid beetles were not described. Poll (1932 *a, b*) described the Malpighian tubules of Buprestid beetles and mentioned that the cell of leptophragma is absent in this group. In the present observations, different Buprestids like *Sternocera* and *Chrysochroa* have been observed and it has been found that the cell forming leptophragma which has been described earlier by Saini in other cryptonephric Coleptera is also present in Buprestid beetles. The cell is similar to the flat leptophragma cell which has been described in Chrysomelidae.

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1. Poll, M., *Rec. Inst. Zool. Torley-Rousseau*, 1932 *a*, 4, 47.
2. —, *Congr. Internation. D'Entomologie Paris*, 1932 *b*, p. 18.
3. Saini, R. S., *Trans. R. Ent. Soc. London*, 1964, 116, 347.

REVIEWS AND NOTICES OF BOOKS

Information Sources in Science and Technology. By C. C. Parker and R. V. Turley. (Butterworths, London), 1975, Pp. 223. Price £ 5.50.

Few of the "broad survey" books on Information Sources that are now flooding the market focus with clarity, thoroughness and wide scope as does the present one under review. The book consists nine chapters on various topics like, defining the problem, choosing sources of information and their guides, literature searching, obtaining literature in usable form, evaluating and storing information, communicating information, current awareness, etc., which are of interest to practising scientists in their fields of interest.

In the preface, the authors write "there is nothing new about most of the factual information contained in these pages. However, we do claim that there is a certain novelty about its presentation, and therein lies our justification for producing yet another contribution to the literature explosion. We have tried to make this book easy to use by arranging its contents in a logically structured fashion, with more or less self-contained sections. This is no more than an introductory guide; nor is it in any sense 'Complete'. We must emphasise that we have produced only a "selection"; Comprehensiveness is not to be looked for in a book of this size". Judged by this statement, the authors have covered all the important literature we are aware of and much that we have not read.

Everyone with access to this book will have at his finger tips a wealth of factual information covering an immensely far-reaching selection of information sources and their guides. All data are presented in a straight-forward and easy to use format. The book is unrivalled in its exceptionally clear presentation, its comprehensive charts; its extensive references which direct the reader instantly to his relevant sources, and its coverage of sources outside the literature.

Books about reference sources must be up-dated to retain their value. The authors have added several new sources in this look. Of special interest is the inclusion of a few general interest, computor based, current awareness and Information Retrieval Sources.

We not only recommend this book for all practising scientists and Engineers, especially students of these subjects but we feel that it will serve as a source book to all Librarians and Information Officers, particularly those working in a scientific and industrial environment.

For all practical purposes, this should be a one sentence review: BUY THIS BOOK. Having said this, there is little more to say.

The printing and the get-up of the book are good and in keeping with the traditions of the Publishers. The Reviewers however feel that greater care should have been taken in dealing with the proofs, since a few typographical errors have escaped attention. Eg: In the "Preface", at the end of the first paragraph, the last line which reads "and even Library School Students might derive some benefit from *is* approach (though this book is not expressly written for them)", does not make full meaning of the sentence.

The weakest feature of the book is its astonishingly high price, which will probably limit its purchase to a small number of libraries and specialists.

T. K. S. Iyengar.
G. S. R. Rao.

A Dictionary of Thermodynamics. By A. M. James. (Macmillan Press, Ltd.), 1976. Pp. xi + 262. Price £ 12.00.

This dictionary is intended for use by undergraduate students as well as practising scientists. It provides an elementary treatment of the subject wherein applications of thermodynamic concepts are treated in a simple manner. The book is more of a ready-reckoner rather than a textbook. Entries have been arranged in alphabetical sequence with cross-references where more than one recognised name exists. Mathematical derivations have been avoided. Definitions of quantities and tabulations of useful data have been provided adequately. SI units have been used throughout.

In these days of information explosion and textbook manufacture, such dictionaries do serve a useful purpose. College students and teachers as well as practising scientists will find this dictionary useful. Unfortunately, the book is enormously expensive for its size. It is hoped that this dictionary will be produced in a low-cost edition in this country to enable ordinary citizens to own a copy.

C. N. R. Rao.

Encyclopedia of Environmental Science and Engineering. Edited by J. R. Pfafflin and E. N. Ziegler. (Gordon and Breach, Science Publishers, Inc., One Park Avenue, New York, N.Y. 10016), 1976. Volumes 1, A–M, and 2, N–Z. Pp. xvi + 600 + 491. Price : Vol. 1, £ 41.10, Vol. 2 £ 35.40 (Both Vols. £ 68.60).

The human environment, its pollution and control have, in recent years, been subjects of increasing interest as well as of great concern. Thoughtful writers have been pointing out the pollutional effects, *e.g.*, Rachel L. Carson in her book, *The Silent Spring*, which made people think about the way our environment is being damaged and destroyed.

At the same time a great deal of work has been proceeding on the control of pollution of water, air and soil. The recent work on the control of pollution of the Thames River is indeed a remarkable achievement. While this work indicates great possibilities, the pollution of water, air and soil in and around many industrial and urban areas remains to be controlled.

In this context the publication of two volumes of *Encyclopedia of Environmental Science and Engineering* is commendable. The major purpose of this publication, as stated in the editors' preface, is to bring the contributions of key environmental areas into a single readily available work. The *Encyclopedia* covers A to Z aspects of air, water, soil, pollution, public health, including psychological aspects of man's environment, ecology, environmental education, urban planning and environmental law.

The subjects more extensively dealt with are air pollution, water, waste water, industrial wastes and some general aspects of microbiology, the terrestrial system, process fundamentals and statistical methods for environmental science. The different aspects of air pollution considered are meteorology sources, automotive pollution, effects of atmospheric pollutants, energy sources, control of gaseous pollutants, noise, particulates, remote detection, sulphur removal, stack sampling and urban pollution modelling. The aspects of water and waste water include the chemistry, properties and structure of water, groundwater, estuarine water quality, pollution effects on fish, desalination, water treatment, physical, chemical and biological treatment of waste water, water reuse and recycling of waste materials.

Valuable information is also given on management of solid waste, composting, management of radioactive waste, radiation ecology, occupational health, pesticides, sediment transport and erosion, disinfection and chemosterilization and environmental health administration.

In 1884, Duclaux ascribed decomposition of organic matter in all cases to the action of microorganisms which, as he stated, "are the important, almost the only, agents of universal hygiene". Since then, and notably in recent years, evidence has been accumulating on the occurrence and activity of more specific microorganisms in the oxidation of organic matter in water, soil and in artificial systems of waste water treatment. It would have been more useful if this evidence was also considered in the articles bearing on microbiology and biological treatment of waste water.

Nevertheless the volumes under consideration give a comprehensive account of environmental science and engineering, with numerous text-figures, graphs, line diagrams, photographs and tables of results, which will be very useful to the scientists, engineers and others interested in the study and control of environmental pollution.

S. C. Pillai.

6391/77. Published by S. R. S. Sastry, Manager, Current Science Association, Bangalore-560006
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METAMORPHIC HISTORY OF SAUSAR ROCKS IN THE NORTHEASTERN PART OF THE NAGPUR DISTRICT, MAHARASHTRA

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ABSTRACT

The Precambrian rocks of the Sausar Group occurring in the northeastern part of the Nagpur district have a polyphase structural history and have undergone Barrovian type of regional metamorphism with sillimanite-almandine assemblages. Textural evidence is given here to show that metamorphism in the area has been a polyphase event involving three main episodes of metamorphic crystallisation which have occurred before, during and after the second (F_2) fold phase. The metamorphic climax, marked by the growth of sillimanite in pelitic rocks, is shown to have occurred following the second (F_2) fold movements. Retrogressive mineral growth is shown to have begun with the incidence of the third (F_3) fold movements in the area.

INTRODUCTION

THE Precambrian metamorphic rocks of the Sausar Group underlie a fold belt about 130 miles long and ranging in width upto 16 miles, that extends from Balaghat in the east to Chhindwara in the west. The rocks show Barrovian type of regional metamorphism with Kyanite-sillimanite sequences in the higher grades, the grade of metamorphism gradually increasing from southeast to northwest over the belt. The area concerning the present study is situated in the middle part of the Sausar belt covered by the Reserved Forests of Chorbaoli and Deolapar (one inch sheets 55, 0/6, 7) in the northeastern part of the Nagpur District, Maharashtra (Fig. 1). It falls within the sillimanite zone of the regional scheme of metamorphic zones suggested by earlier workers (Narayanaswami *et al.*[7]. Sausar rocks over this part of the belt have been affected by at least three fold phases (F_1–F_3) and have undergone metamorphism of the almandine-amphibolite facies with sillimanite-almandine assemblages developed in pelitic rocks (Agrawal[1-3]. The purpose of the present investigation has been to study the variation in metamorphic grade in time in the above rocks. This has been achieved by determining the periods of growth of successive metamorphic (index) minerals in relation to the established sequence of structures (F_1–F_3) in the pelitic rocks which are considered to be the most sensitive material to temperature changes. Minor structures related to various fold phases have been employed as 'time-markers', and the growth of porphyroblastic minerals dated with respect to them. The technique used has been described earlier by Rast[9], Zwart[11,12], Sturt and Harris[10], Johnson[5,6] and Harte and Johnson[4].

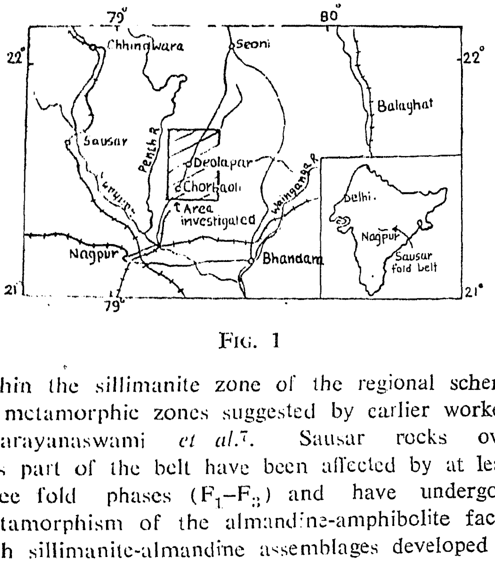

FIG. 1

TIMES OF FORMATION OF MINERALS

Development of Micas

Micas have first developed as tiny flakes during the initial fold movements, defining the primary schistosity (S_1) axial planar to first (F_1) fold structures. This is revealed by the fine grained schistose fabric related to F_1 still preserved as ei's in pre-F_2 porphyroblasts. Recrystallisation of micas has occurred during the second (F_2) fold phase, as suggested by the occurrence of relatively coarser flakes of biotite and muscovite with (001) aligned on axial planes of minor F_2 structures in pelitic schists. In thin sections of some specimens of pelitic schists large biotite flakes are found to be arranged on the hinges of F_2 crenulations in a herringbone pattern, without showing any sign of bending or breaking, which suggests that biotite has continued to grow in post-F_2 period mimetically on F_2 fold hinges. As observed today, micas defining the primary regional schistosity appear identical with those aligned on axial planes of minor F_2 structures and with unstrained micas occurring on hinges of F_2 crenulations. This suggests that the initial fine grained schistose fabric that developed during the formation of F_1 structures, has been modified subsequently by recrystallisation and growth of micas during F_2-post-F_2 period. This has also been accompanied by a general coarsening of the matrix fabric.

Specimens of Mansar schists from Deolapar show the development of porphyroblastic muscovite flakes overgrowing the second schistosity (S_2). While micas defining S_2 are affected by F_3 crenulations, the porphyroblastic muscovite shows no sign of strain, which suggests that the latter may have developed during or after F_3 movements.

Development of Garnet

Garnet is a common constituent of pelitic schists of Mansar and Junawani formations. It occurs frequently in biotite gneisses and, occasionally, in granulites of Kadbikhera formation. Inclusions of quartz, iron ore and micas are common in garnets. Elongate inclusions of these minerals, mostly arranged in straight trails across the porphyroblasts of garnet, define a fine grained Si (internal schistosity) which is usually oriented at a high angle to Se (matrix schistosity) without showing any continuity with the latter. Large flakes of biotite and muscovite defining Se abut directly against the boundaries of porphyroblasts, enclosing at the same time smaller grains of garnet. The inclusions forming Si's are considerably smaller than grains of the same minerals in matrix schistosity (Se), indicating that garnets have grown in a schistose fabric that was finer grained than the matrix schistosity. Garnets, developed in Mansar schists of Deolapar, occasionally contain symmetrically curved trails of inclusions which are finer grained than the same minerals in Se. The curved Si's, like straight Si's, are usually oriented at an angle to Se without any continuity with the latter. The Si–Se relations suggest that garnets have grown mainly in the static period between the first (F_1) and second (F_2) fold movements. In Deolapar, garnet growth seems to have begun before the first (F_1) fold movements had ceased, thereby indicating that higher temperatures may have prevailed in the northern part of the area at the time.

Development of Sillimanite

Sillimanite is developed in pelitic schists of Mansar and Junawani formations and also in biotite gneisses. Fibrolite, occurring as bundles of fibres, is more common, although long slender crystals are also developed in places. Fibrolite is usually associated with the second schistosity (Se). It shows preference for reddish brown biotite in its site of nucleation, and there is clear evidence of its growth at the expense of the latter. Relics of biotite are found in mats of fibrolite in most of the thin sections from Mansar schists. In some cases merely pleochroic halos are visible in pools of fibrolite. F_3 crenulations which deform the micas of second schistosity (S_2) also seem to affect the fibrolite. The biotite-fibrolite relationship suggests that the growth of fibrolite has occurred in the static period between the second (F_2) and the third (F_3) fold movements.

Retrogressive Mineral Growth

It the area investigated there is evidence of limited retrograde metamorphism. Pelitic schists show retrogressive alteration of biotite and garnet to chlorite in the beginning stages. The concentration of retrogressive chlorite in zones of F_3 crenulations seems to suggest that the retrogressive change began with the incidence of the third (F_3) fold movements.

RELATIONS BETWEEN METAMORPHISM AND DEFORMATION

The time relationships of structural and metamorphic episodes in the area under consideration are summarised in Table I. The results show that the metamorphism in the area has been a polyphase event involving three main episodes of metamorphic crystallisation that have occurred before, during, and after the second (F_2) fold phase. At each successive stage of metamorphic crystallisation a higher grade mineral assemblage has developed which suggests that the metamorphism has been progressive in time. The final fabric of the metamorphic rocks is the end product of interactions of successive metamorphic and structural events and may be regarded as a 'composite fabric'.

In the initial stages of deformation, metamorphic conditions in the range of biotite grade, probably of a higher grade in the northern part, seem to have prevailed in the area, which have favoured the development of folds (F_1) of 'similar' type (Class 2, *cf.* Ramsay[8]), accompanied by an axial plane schistosity (S_1). This has been followed by a static (post-F_1 and pre-F_2) episode of metamorphism during which time porphyroblasts of garnet have developed in pelitic schists throughout the area, enclosing the fine grained schistose fabric related to first (F_1) fold phase as inclusion trails (Si's). With the incidence of the second (F_2) fold movements the earlier schistosity (S_1) has been deformed, accompanied by rotation and deformation of enclosed porphyroblasts of garnet. A new schistosity (S_2) axial planar to second fold structures has developed, defined by large flakes of biotite and muscovite which have recrystallised synkinematically with the second (F_2) fold phase. F_2 folds range in style between Class 2 and Class 1*c* (*cf.* Ramsay[8]). The widespread recrystallisation and growth of micas that began in the second (F_2) fold phase, marking the Syn–F_2 episode of metamorphism, and probably outlasted it, has transformed the fine-grained schistose fabric related to F_1 into a coarse schistosity. The

TABLE I

Periods of mineral growth in relation to the structural time-scale in psammitic-pelitic rocks of the Sausar group in Chorbaoli-Deolapar area, Nagpur District, Maharashtra

Structural Episodes	Metamorphic episodes	
	Kadbikhera formation	Mansar/Junawani formations
F_1 Overturned Isoclinal to Recumbent folds (= Deolapar Nappe) with E–W to ESE–WNW axial planes	Biotite and muscovite	Biotite and muscovite Synkinematic growth of garnet (in Deolapar)
	Post-kinematic growth of garnet (almandine)	Post-kinematic growth of garnet (almandine)
F_2 Close to tight asymmetric folds with E–W to ESE–WNW axial planes	Synkinematic to post-kinematic growth of biotite/muscovite	Synkinematic to post-kinematic growth of biotite/muscovite
	A GENERAL COARSENING OF THE MATRIX FABRIC	
		Post-kinematic growth of Sillimanite (breakdown of biotite)
F_3 Open upright folds with NNE–SSW axial planes	Retrograde metamorphism Garnet → } Chlorite Biotite → } Chlorite	Retrograde Metamorphism Garnet → } Chlorite Biotite → } Chlorite

residual F_1 fabric is preserved only as Si's in pre-F_2 porphyroblasts. Not only micas have grown during F_2-post-F_2 period, but a general coarsening of the mineral grains making up the matrix schistosity (Se) about pre-F_2 garnet porphyroblasts has occurred during that period. Metamorphism has reached a climax in the static period between the second (F_2) and the third (F_3) fold phase, marked by breakdown of S_2-biotite and growth of sillimanite. By the time of incidence of the third (F_3) fold phase temperatures started declining as suggested by the style of fold structures of this generation, which approximate to Class 1B (*cf.* Ramsay[8]) and are associated with fracture cleavage marking the beginning of brittle deformation. The concentration of retrogressive chlorite in zones of F_3 crenulations in pelitic schists suggests that retrograde metamorphism may have begun with the incidence of the third (F_3) fold phase.

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1. Agrawal, V. N., "The structure and genesis of the manganese ores and associated rocks of the Sausar series around Chorbaoli, Nagpur District, Maharashtra, Univ. Saugar", *Ph.D. Thesis*, 1968 (Unpublished).
2. Agrawal, V. N., *Proc. Ind. Nat. Sci. Acad.*, 1974, 40 A (2), 101.
3. —, *J. Geol. Soc. Ind.*, 1975, 16 (2), 176.
4. Harte, B. and Johnson, M. R. W., "Metamorphic history of Dalradian rocks in Glens Clova, Esk and Lethnot, Angus, Scotland," *Scott. J. Geol.*, 1969, 5 (1), 54.
5. Johnson, M. R. W., "Relations of movement and metamorphism in Banffshire," *Trans. Edin. Geol. Soc.*, 1962, 19, 29.
6. —, "Some time relations of movement and metamorphism in the Scottish Highlands," *Geol. Mijnb.*, 1963, 42, 121.
7. Narayanaswami, S. *et al.*, "The geology and manganese ore deposits of the Manganese Belt in Madhya Pradesh and adjoining parts of Maharashtra," *Bull. Geol. Surv. Ind.*, Ser. A—Econ. Geol., 1963, Pt. 1.
8. Ramsay, J. G., *Folding and Fracturing of Rocks*, New York, 1967.
9. Rast, N., "Metamorphic history of the Schichallion Complex, Perthshire," *Trans. R. Soc. Edin.*, 1958, 63, 413.
10. Sturt, B. A. and Harris, A. L., "The metamorphic history of the Loch Tummel area, Central Perthshire, Scotland," *Lpool Manchr. Geol. J.*, 1961, 2, 689.
11. Zwart, H. J., "Relations between folding and metamorphism in the Central Pyrenees and their chronological succession," *Geol. Mijnb.*, 1960, 39, 163.
12. —, "On the determination of polymetamorphic mineral associations and its application to the Bosost area (Central Pyrenees)," *Ibid.*, 1962, 52, 38.

MOLECULAR ORBITAL STUDIES ON THE CONFORMATION OF N-PHENYL MALEIMIDE

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SUBSTITUTED maleimides have found several biological and technical applications[1-8]. They have been used as heat stabilisers and thermal discoloration inhibitors in polymers, adhesives for metals, vulcanization agents, fungicides and choline transfer inhibitors to mention a few. Such compounds form complexes with oxy and deoxy hemoglobin[5-7] and are reactive to biological species[8]. Due to such a wide variety of interests, it was thought worthwhile to investigate the conformation of a substituted maleimide, namely N-phenyl maleimide. It may be mentioned that NMR investigations on a related molecule N-*p*-chlorophenyl maleimide have been reported[9]. From the analysis of the NMR spectrum of the molecule oriented in a nematic solvent, it was found that there are only two nonequivalent dipolar couplings [$D(i, j)$ as defined in literature[10]] between phenyl and the maleimide protons, *i.e.*, D(H3, H7) = D(H4, H7) = D(H3, H11) = D(H4, H11) and D(H3, H8) = D(H3, H10) = D(H4, H8) = D(H4, H10). Two possible interpretations can satisfy the NMR observations: (*a*) a rapid exchange between planar and orthogonal conformations with appropriate weighting factors and (*b*) a twisted conformation with C_2 symmetry for the molecule with a rapid exchange between the four equivalent conformers.

Since both the rings are planar, the conformational structure of N-phenyl maleimide is determined by a single torsional angle α around the C–N bond. We choose a convention in which the 0° for α corresponds to the conformation in which both the rings lie in the same plane. Two semi-empirical molecular orbital methods namely, CNDO and PCILO[11,12], were used for the calculation of total energy (V) of the molecule as a function of angle α. Both these methods have been extensively used for conformational energy calculations and found quite successful for predicting conformational structure[13-16]. Standard bond lengths and bond angles (shown in Fig. 1.) were used to fix the primary geometry of this molecule. The results of the energy calculations are also included in the figure. The energy curves show a four-fold behaviour, because of the molecular symmetry. For this reason only 0–90° section has been shown in the figure. It is found that both the planar and orthogonal ($\alpha = 90°$) conformations correspond to maxima in the energy profiles. Energy curves from both the methods are parallel to one another and show minima at an angle of about 25°. The potential energy curve is relatively flat in the region of 0–40° but the energy rises sharply as the conformation tends towards an orthogonal arrangement of the two rings. The four equivalent minima are connected by barrier heights which are low on the NMR time-scale. The theoretical results reported here clearly exclude the possibility of an equilibrium between planar and orthogonal conformations, but are consistent with the alternative interpretion of the NMR results and suggest a twisted conformation with a low barrier for rotation around C–N bond.

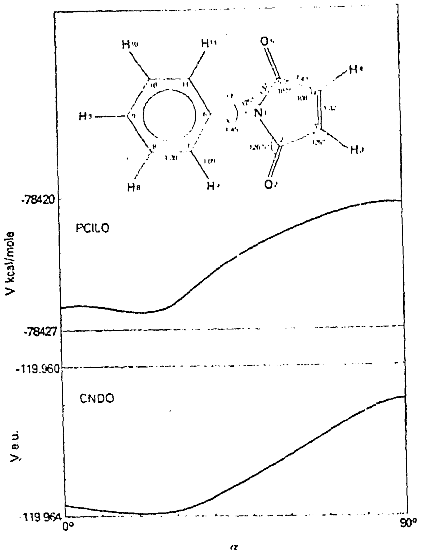

FIG. 1. Potential energy (V) of N-Phenyl maleimide as a function of torsional angle α.

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1. Juergen, R. and Wally, N., *Faser Forsch. Textiltech.*, 1970, **21**, 105.
2. Hiroshi, A., Teruo, H., Katsushige, Y. and Yoshiaki, S., *Fukui Daigaku Kogakubu Kenkyu Hokoku*, 1969, **17**, 55.
3. Nudelman, Z. N. and Butyagina, N. D., *Kauch Rezima*, 1970, **29**, 7.
4. Mannervik, B. and Sorbo, B., *Biochem. Pharmacol.*, 1970, **19**, 2509.

5. Simon, S. R., Arndt, D. J. and Konigsberg, W. H., *J. Mol. Biol.*, 1971, **58**, 69.
6. Moffat, J. K., *Ibid.*, 1971, **58**, 79.
7. —, Simon, S. R. and Konigsberg, W. H., *Ibid.*, 1971, **58**, 89.
8. Potter, R. L. and Hadley, M. E., *Experientia*, 1970, **26**, 536.
9. Khetrapal, C. L., Kunwar, A. C. and Saupe, A., *Mol. Cryst. Liq. Cryst.* (to be published).
10. Khetrapal, C. L., Kunwar, A. C., Tracey, A. S. and Diehl, P., In: *NMR Basic Principles and Progress*, Springer-Verlag, Berlin, 1975, **9**, 1.
11. Diner, S., Malrieu, J. P. and Claverie, P., *Theor. Chim. Acta*, 1969, **13**, 1.
12. Pople, J. A. and Segal, G. A., *J. Chem. Phys.*, 1966, **44**, 3289.
13. Pullman, B. and Pullman, A., *Adv. Protein Chem.*, 1974, **16**, 347.
14. Govil, G. and Saran, A., *J. Chem. Soc.*, 1971, p. 3624.
15. — and —, *J. Theor. Biol.*, 1971, **33**, 391.
16. Gupta, S. P., Govil, G. and Mishra, R. K., *Ibid.*, 1975, **51**, 13.

HIGH ENERGY DOUBLE PLASMON SATELLITE OF $L_{2,3}$ EMISSION BAND OF Na

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ABSTRACT

Relative intensity of high energy double plasmon satellite in the $L_{2,3}$ X-ray emission spectrum of Na has been calculated as 0·01. The calculated value is in excellent agreement with observed value of Arakawa and William (*Z. Physik*, 1972, 251, 271)

RECENTLY Hanson and Arakawa[1] have studied the high energy X-ray satellites of $L_{2,3}$ emission bands of Na, Mg, Al and Si. They have reported a high energy satellite of Na $L_{2,3}$ emission band at an energy distance of (11·3 ± 0·5) eV from the main band and have attributed the origin of this satellite as due to double ionization process and excluded the possibility of plasmon energy gain process.

However, if we look at the Table I of Hanson and Arakawa[1], we see that the energy separation (11·3 ± 0·5) eV of high energy satellite in Na corresponds well with double plasmon energy ($2\hbar\omega_p \approx 11 \cdot 4$ eV) for Na. This energy correspondence suggests that this satellite may be due to double plasmon energy gain process about which nothing has been said so far. Since Hanson and Arakawa have assigned the above satellite as due to double ionization process, it was thought of great interest to calculate the relative intensity of the above satellite on the basis of double plasmon energy gain process and to compare the same with the observed value of Hanson and Arakawa[2].

Several workers[3-6] have studied theoretically the involvement of plasmons in the emission of high energy satellites at an energy distance of $\hbar\omega_p$ from the parent band. These satellites have been observed[1] in Al and Si in X-ray emission and in Auger Spectrum[7-9], of Al, Mg, Si and Be. Recently the authors[10] have shown the possibility of double plasmon energy gain in Auger spectrum of Be.

Following Langreth[11,12], the relative intensity of double plasmon satellite with the main band can be expressed as

$$i = \frac{0 \cdot 06\, a^2}{2\, e^{-\alpha}} \tag{1}$$

where

$$\alpha = \frac{e^2 q_c}{\pi\hbar\omega_p} \approx 0 \cdot 12\, r_s.$$

(r_s-being dimensionless parameter in Bohr's unit)

The relative intensity of high energy satellite of $L_{2,3}$ emission band of Na at about 11·3 eV has been calculated from equation (1) taking the value[13] of $r_s = 4$, as 0·01 which is in excellent agreement with the observed value 0·01 of Hanson and Arakawa (*see* Table I).

TABLE I

Metal	$\hbar\omega_p$ [11] (eV)	$2\hbar\omega_p$ (eV)	ΔE^1 (Obs.) (eV)	Relative intensity	
				Authors' value	Hanson and Arakawa[2] value
Na	5·7	11·4	(11·3 ± 0·5)	0·01	0·01

Thus from energy separation as well as relative intensity point of view the high energy satellite in Na may be regarded as due to double plasmon energy gain process.

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1. Hanson, W. F. and Arakawa, E. T., *Z. Physik*, 1972, 251, 271.

2. Arakawa, E. T., Private Communication.
3. Shmidt, V. V., *Soviet Physik. JETP*, 1961, 12, 886.
4. Steimann, W., *Phys. Stat. Sol.*, 1968, 28, 437.
5. Srivastava, K. S., Singh, S. P. and Shrivastava, R. L., *Phys. Lett.*, 1974, 47 A, 305.
6. Neddermeyer, H. and Wiech, G., *Ibid.*, 1970, 30 A, 17.
7. Jenkins, L. H. and Chung, M. F., *Surf. Sci.*, 1971, 28, 409 and 1972, 33. 159.
8. Chung, M. F. and Jenkins, L. H., *Ibid.*, 1971, 26, 649.
9. Jenkins, L. H., Zehner, D. M. and Chung, M. F., *Ibid.*, 1973, 38, 327.
10. Srivastava, K. S., Shrivastava, R. L. and Harsh, O. K., *Curr. Sci.*, 1977, 46 (1), 5.
11. Langreth, D. C., "Collective properties of physical system," *Noble*, 1973, 24, 210.
12. —, *Phys. Rev. Lett.*, 1971, 26, 1229.
13. *Soft X-ray Band Spectra*, Edited by D. J. Fabian, Academic Press, p. 8.
14. Sutherland, J. C., Arakawa, E. T. and Hamm, R. N., *J. Opt. Soc. Am.*, 1967, 57, 645.

HYDROXY TRITERPENOIDS—I

Conversion of Methyl Oleanonate to Methyl 2α, 3β-Dihydroxyolean-12-Ene-28-Oate and 2α, 3β, 28-Trithydroxyolean-12-Ene and their Epimers

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ABSTRACT

Methyloleanonate (I) is converted to methyl 2α, 3β-dihydroxyolean-12-cne-28-oate (IV *a*), its 2β-epimer (V), 2α, 3β, 28-trihydroxyolean-12-ene (VI *a*) and its 3α-epimer by acetoxylation with Pb (OAc) 4 followed by reduction with metal hydrides.

HYDROXYLATION in ring A of triterpenoids is accomplished by four methods, (i) reaction of olean-2-enes with OsO_4[1], (ii) hydroboration of the enol-3-acetates followed by oxidation with H_2O_2[2], (iii) reaction of the enolisable ketones with Pb $(OAc)_4$ followed by reduction with metal hydrides[3], and (iv) autooxidation of C-3 ketones to the diosphenols followed by catalytic hydrogenation and reduction[4]. We now report the partial synthesis of methyl 2α, 3β-dihydroxyolean-12-ene-28-oate (IV *a*), its 2β-epimer (V), 2α, 3β, 28-trihydroxyolean-12-ene (VI *a*) and its 3α-epimer from methyloleanonate (I).

Treatment of methyloleanonate (I) with Pb $(OAc)_4$ in glacial acetic acid at 100° or in benzene using $BF_3.(OC_2H_5)_2$ as catalyst gave a product which showed three spots on tlc. Crystallisation of the crude product from methanol afforded the 2α-acetoxy-3-ketone (II), m.p. 224–25° (lit.[7] m.p. 226–28°), $(\alpha)_D + 65°$ in about 30% yield. In this reaction the acetoxyl group approaches from the less hindered rear face of the molecule. Its IR and ^1H NMR spectral data [ν_{max} 1750 (ester C=O), 1720 (ring C=O) cm^{-1}; δ 5·57 a pair of doublets $J_{a,a} = 13$ Hz, $J_{a,e} = 6$ Hz, H–2] suggest an equatorial conformation for the acetoxyl at C–2 in II. The acetoxy ketone (II), when adsorbed on basic alumina, was found to rearrange to the 2-oxo-3β-acetate (III), m.p. 190–92° (lit.[7] m.p. 197–98°), $(\alpha)_D + 105°$. From the spectral data (ν_{max} 1745, 1720 cm^{-1}; δ 4·95 s, H–3), it is inferred that the acetoxyl at C–3 is equatorial. A precedent for this isomerisation is found in the work of Ganguli *et al.*[4] who suggested a plausible mechanism.

Reduction of the acetoxy ketone (II) with $NaBH_4$ in methanol gave in high yield methyl maslinate (IV *a*), m.p. 228–30° (lit.[5] m.p. 227–28°), $(\alpha)_D + 75°$; diacetate (Ac_2O-pyridine), m.p. 175–77° (lit.[5] m.p. 166–68°), $(\alpha)_D + 39°$. The ^1H NMR spectral data of the diacetate (IV *b*) (δ 4·75 *d*, J = 11 Hz, H–3, 5·05 qd, H-2) suggest that H–2 and H–3 are *trans* diaxial and the hydroxyls in IV *a* are therefore *trans* diequatorial[3,6]. Similar reduction of the ketone (III) with $NaBH_4$ afforded the *cis* diol (V), m.p. 268–73° (lit.[7] m.p. 278–80°), $(\alpha)_D + 72°$ in high yield (70%).

The 2α-acetoxy-3-ketone (II) on reduction with $LiAlH_4$ in dry ether gave a mixture of two compounds (tlc), the olean-12-ene-2, 3, 28-triols, which were separated by chromatography on acid washed alumina and purified by preparative tlc. The more polar triol (75% yield) is identified as 2α, 3β, 28-trihydroxy-olean-12-ene (VI *a*), m.p. 272–76°, (lit.[9] m.p. 275–79°), $(\alpha)_D + 70°$, ν_{max} 3400 cm^{-1}; δ 3·65 m, H–2; 3·00 d, J = 10 Hz, H–3. The ^1H NMR spectrum of its triacetate (Ac_2O-pyridine) m.p. 183–85°, (lit.[9] 187–88°) $(\alpha)_D + 20°$, (δ 3·90 AB 5, J = 10·5 Hz,-CH_2-OAc; 5·00 m, H–2; 4·77 d, J = 10·5 Hz, H–3) indicated that the hydroxyls in ring A of the triol (VI *a*) possess *trans* diequatorial orientation. The less polar triol (yield 15%), m.p. 245–50°, $(\alpha)_D + 45°$ is probably the 3-epimer of VI *a*. Its ^1H NMR data (δ 4·10 m, $W_{1/2h}$=22 Hz, H–2β; 3·45m, $W_{1/2h}$=4 Hz resolved as doublet J=4 Hz, H–3β; 3·40 AB q J=11Hz, $-CH_2OH$) suggest that H–2 and H–3 are axial and equatorial respectively[8]. There is so far no report in literature on the natural occurrence of these triols and it is likely that they may be found along with the corresponding C-17 carboxylic acids.

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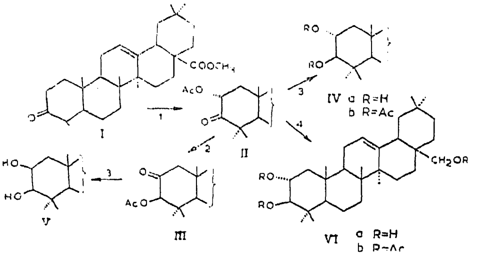

1. $Pb(OAc)_4$-HOAc or $Pb(OAc)_4$-$BF_3.(OC_2H_5)_2$.
2. Basic Al_2O_3.
3. $NaBH_4$–CH_3OH.
4. $LiAlH_4$–dry ether.

All compounds reported in this paper gave satisfactory C and H analyses. IR spectra were taken in KBr on Perkin Elmer Model 137 spectrophotometer and ^1H NMR spectra in $CDCl_3$ (MeS_4i as internal standard) on Varian A-60 instrument. Optical rotations were measured in methanol solution at room temperature.

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1. Djerassi, C., Thomas, D. B., Livingston, A. L. and Thomson, C. R., *J. Am. Chem. Soc.*, 1957, **79**, 5292; Tschesche, R., Henkel, E. and Snatzke, G., *Ann.*, 1964, **676**, 175.
2. Caglioti, L. and Cainelli, G., *Tetrahedron*, 1962, **8**, 1061.
3. Boul, A. D., Fairweather, P. M., Hall, J. M. and Meakins, G. D., *J. Chem. Soc.* (C), 1971, p. 1199; Row, L. R., Rukmini, C. and Rao, G. S. R. S., *J. Ind. Chem. Soc.*, 1963, **40**, 311.
4. Ganguli, A. K., Govindachari, T. R. and Mohammad, T. A., *Tetrahedron*, 1966, **22**, 3597.
5. Sengupta, P. and Bikramdas, P., *J. Ind. Chem. Soc.*, 1965, **42**, 537.
6. Cheung, H. T. and Fang, M. C., *J. Chem. Soc.* (C), 1968, p. 1045.
7. Bannon, C. D., Eade, R. A., James, P. and Simes, J. J. H., *Austr. J. Chem.*, 1973, **26**, 629.
8. Cheung, H. T. and Fang, M. C., *Chem. Comm.*, 1970, p. 369. Jackman, L. M. and Sternhill, S., *Applications of NMR Spectroscopy in Organic Chemistry*, Pergamon Press, 1969, p. 280.
9. Glen, A., Lawrie, W., McLean, J. and Garby Younes, M. El., *J. Chem. Soc.* (C), 1967, p. 510.

PROF. G. N. RAMACHANDRAN, F.R.S.

Professor G. N. Ramachandran, who has been recently elected a Fellow of the Royal Society, was a student of Prof. Sir C. V. Raman, with whom he worked for his doctorate at the Indian Institute of Science, Bangalore. After this, he went to the University of Cambridge, England, where he worked with Prof. Sir Lawrence Bragg from 1947 to 1949. He has been the Professor and Head of the Department of Physics at the University of Madras from 1952 to 1970 where he initiated work on the structure of biological macromolecules, studied essentially by methods of X-ray diffraction. This work initially was centred on the protein collagen, which forms the essential constituent of the connective tissue (skin, tendon, bone, etc.). Prof. Ramachandran has also been one of the earliest persons in the world to start studies on molecular bio-physics—in particular, on protein conformation and the structures of other biological macromolecules. In this, he and his colleagues introduced the method "dihedral angles" for working out the full three-dimensional structures of these biopolymers and related compounds.

In addition to being a Fellow of the Indian Academy of Sciences and Indian National Science Academy, Prof. Ramachandran is an Honorary Member of the American Society of Biological Chemists, an Honorary Foreign Member of the American Academy of Arts and Sciences and also a Fellow of the Royal Society of Arts, London. He has edited 8 volumes on different aspects of biopolymer structure, and has written two books on crystallography. He was the Editor of *Current Science* from 1951 to 1957, and has been, on the Editorial Board of several International Journals.

During the last six years at the Indian Institute of Science, he has set up a Department of Molecular Biophysics, which is working on the whole range of biopolymers, using a wide range of techniques. Among the achievements of this Laboratory are the finishing touches to the triple-helical structure of collagen—the role of hydroxyproline in this protein—and the development of a new method of solving crystal structures using packing considerations. They are also working on a new structure of DNA, which is rather different from the well-known Watson-Crick double helix. Among other important studies are those on protein-polysaccharide complexes, on membranes and on antibiotics.

LETTERS TO THE EDITOR

DIRECTIONAL CORRELATION OF THE 404-GAMMA-216 GAMMA CASCADE IN ^{131}Cs

RECENTLY Sharma *et al.*[1] reported the gamma-gamma directional correlation measurements in ^{131}Cs from ^{131}Ba using Ge (Li)-NaI (Tl) spectrometer combination and fast coincidence set up. From their 404 γ–216 γ directional correlation coefficients as listed in Table I they obtained for the 216 keV level a spin of $3/2^+$ or $5/2^+$ and the 404 keV transition as M1 + (98·5 ± 0·5) % E2 considering the 620 keV level as $3/2^+$. In support of this they have cited that Kucarova *et al.*[2] and Singh *et al.*[3] obtained 487 and 620 keV transitions as M1 + E2 from the internal conversion coefficient measurements. However, we found that the A_{22} coefficient for this cascade as obtained by Sharma *et al.*[1] was much smaller than the one obtained by Sturm *et al.*[4] using NaI (Tl) detectors. Noting this wide difference the directional correlation coefficients have been measured using two 2″ × 2″ NaI (Tl) detectors and a fast coincidence set up having a resolving time of 40 nsec. The results are listed in Table I along with the results of Sturm *et al.*[4] The details are given below.

TABLE I

Results of different measurements on the 404γ–216γ cascade and conclusions from the present work

Sl. No.	A_2	A_{44}	Ref.
1	−0·117 ± 0·004	−0·009 ± 0·016	4
2	−0·028 ± 0·019	−0·016 ± 0·028	1
3	−0·122 ± 0·007	−0·004 ± 0·012	Present work

620 keV level: $1/2^+$; $Q_{444} \geqslant 0{\cdot}995$.

From the internal conversion coefficient measurements of Hasselgren *et al.*[5] and Morinaga and Hisatake[6] regarding the K and L subshells of the 216 keV transition, a δ value of 0·107 ± 0·007 has been found for this transition. This gives $A_2(216\gamma) = 0{\cdot}2281 \pm 0{\cdot}0081$. From our A_{22} measurements taking away the $A_2(216\gamma)$ part and analysing $A_2(404\gamma)$ for the evaluation of Q_{404} we find that with 620 keV level having a $5/2^+$ spin no solution exists. The spin of the 216 keV level has been taken as $3/2^+$ as determined by Hasselgren *et al.*[5], Sturm *et al.*[4] and Sahota[7]. A spin of $3/2^+$ for the 620 keV level yields $Q_{404} < 0{\cdot}92$ and $1/2^+$ gives $Q_{404} \geqslant 0{\cdot}995$.

Thus we conclude that the A_{22} coefficient of Sharma *et al.*[1] is definitely too small. The results of the present measurement agree perfectly with the measurements of Sturm *et al.*[4] and confirm the previous spin assignment of $1/2^+$ to the 620 keV level.

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1. Sharma, A. K., Verma, H. R., Bhatti, S. S. and Trehan, P. N., *Nucl. Phys. and Solid State Phys. Symp. (India)*, 1975, **18 B**, 258.
2. Kucarova, T., Kracik, B. and Zvolska, V., *Sov. Jr. Nucl. Phys.*, 1968, 7, 433.
3. Singh, R., Rangacharyulu, C. and Mehta, G. K., *Z. Phys.*, 1973, **258**, 12.
4. Sturm, R., Theile, F. W., Thielmann, R. and Grunberg, E., *Ibid.*, 1972, **252**, 312.
5. Hasselgren, L., Antman, S., Sahota, H. S. and Thun, J. E., *Nucl. Phys.*, 1970, **153 A**, 625.
6. Morinaga, A. and Hisatake, K., *J. Phys. Soc. Jap.* 1975, **38**, 322.
7. Sahota, H. S., *Ind. J. Phys.*, 1976, **50**, **459**.

EFFECT OF CONCENTRATION ON THE FLUORESCENCE OF MERCUROCHROM

MERCUROCHROM, a dibromo mercury substituted derivative of fluorescein, is highly fluorescent. The change in the concentration of this compound in aqueous solution causes a remarkable shift in the excitation and emission peaks in the fluorescence spectrum. A systematic study of these shifts are reported in this note. The emission and excitation spectra are recorded using an Aminco Bowman Spectrophotofluorometer. The concentration of mercurochrom is varied from $7 \cdot 5 \times 10^{-8}$ g/cc to $1 \cdot 5 \times 10^{-4}$ g/cc at room temperature (~35° C). The results are given in Table I.

TABLE I

Concentration g/cc	λ_{ex} (nm)	-λ_{em}(nm)
$7 \cdot 5 \times 10^{-8}$	506	538
$7 \cdot 5 \times 10^{-7}$	508	538
$3 \cdot 75 \times 10^{-6}$	508	538
$7 \cdot 5 \times 10^{-6}$	508	538
$2 \cdot 5 \times 10^{-5}$	520	548
$3 \cdot 75 \times 10^{-5}$	524	552
$7 \cdot 5 \times 10^{-5}$	530	556
$1 \cdot 5 \times 10^{-4}$	538	560

It is seen (Table I) that on increasing the concentration from $7 \cdot 5 \times 10^{-8}$ g/cc to $7 \cdot 5 \times 10^{-6}$ g/cc, the emission peak remains constant at 538 nm but the peak position changes to 560 nm as the concentration is further increased to $1 \cdot 5 \times 10^{-4}$ g/cc. Thus it is seen that the emission peak shows a red shift. From Table I, we can easily infer that (1) the emission wavelength is almost constant over the concentration range $\sim 10^{-8}$ to $\sim 10^{-6}$ g/cc. (2) There is a sharp break in the constant nature of the wavelength at around $\sim 10^{-5}$ g/cc and (3) For further increase in concentration upto $\sim 10^{-4}$ g/cc and the emission wavelength increases linearly. The break in the linear nature around concentration $\sim 10^{-5}$ g/cc can be explained as follows:

Mercurochrom in solution, like fluorescein[1-8] can exist as neutral molecules, mono or dianions, dimers of neutral molecules or of dianions or even polymers. The break in the constant nature of the graph suggests that around this concentration ($\sim 10^{-5}$ g/cc), a change in the molecular form of the dye takes place, *e.g.*, from monomeric to possibly dimeric or even polymeric at higher concentrations. Though there is a possibility of these two varieties existing simultaneously in this investigation, we find only one emission peak and not two for each concentration. This shows that at very low concentrations, the emission is mainly due to the monomeric species while at high concentrations, possibly the dimers are formed and the large change in the emission wavelength is due to this variety.

The excitation peak for maximum emission intensity also shows a similar red shift with increasing concentration. In this case also the discontinuity occurs around concentration $\sim 10^{-5}$ g/cc. This behaviour is similar to that of emission spectra as detailed above.

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1. Machwe, M. K., *Curr Sci.*, 1970, **39** (18), 412.
2. Pant, D. D. and Pant, H. C., *Ind. J. Pure and Appl. Phys.*, 1968, 6, 238.
3. Rathi, S. S. and Machwe, M. K., *Acta Phys. Hung.*, 1967, **23**, 449.

SOLAR ABSORPTANCE PROPERTIES OF SOME NONSELECTIVE COATINGS

AN ideal solar absorptance coating should have maximum absorptance ($\alpha = 1$) over the solar spectrum region ($0 \cdot 25$–$2 \cdot 5$ μm) and minimum emittance ($\epsilon = 0$) in the infrared region ($2 \cdot 5$–30 μm). The spectral reflectance measurements were carried out on some of the Indian paints in the visible and infrared region to asses their performance characteristics.

Three Indian paints, *viz.*, black-board paint (commercial sample A), black paint (commercial sample B) and black enamel paint (commercial sample C) were sprayed on 50 mm × 50 mm × 3 mm thick aluminium substrates to a thickness of few microns. The substrates were cleaned with dilute KOH and rinsed with water and dried before the paint was sprayed. The coatings were allowed to dry at room temperature for more than 6 hours and some were dried at 100° C for a few hours.

The spectral reflectance measurements were carried on a reflectometer constructed in this laboratory in the visible region and Carl Zeiss UR–10 Spectrophotometer for infrared region at an incidence angle of 15°. The reflectance percentage *versus* wavelength between $0 \cdot 45$–16 μm for the three paints are given in Fig. 1.

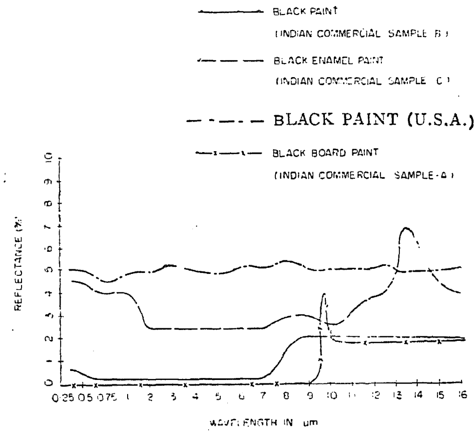

FIG. 1. Spectral reflectance for various paints.

A black paint (U.S.A.) which is specifically made for the application in their solar collector systems production, reflectance curve is also given for comparison[1]. Black-board paint (sample A) gives zero reflectance from $0 \cdot 45$ to 9 μm and then sharply rises to $R = 4 \cdot 5\%$ in a narrow region and gives $R = 2\%$ in infrared region. The black paint (sample B) also gives low reflectance in the solar spectrum. However the behaviour of the black enamel coating (sample C) curve shows higher reflectances in visible and infrared region compared with the other two paints. Black paint (U.S.A.) gives uniformly 5% reflectance in both solar spectrum and infrared region. The black-board paint (sample A) could be graded as most efficient absorbing coating for low thermal solar collectors as it gives zero reflectance in solar spectral region.

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1. Pandit, G. Patil, *Solar Energy*, 1975, 17, 111.

LOW ENERGY PHOTON ATTENUATION COEFFICIENTS

Measurements on total photon attenuation coefficients at low energies are of interest because of their high dependence on energy. Most of the available measurements are carried out either with NaI (Tl) detectors or crystal diffraction gratings. In view of the resolution and energy spread difficulties respectively of the above two systems, it is desirable to undertake measurements on total attenuation coefficients either with Si (Li) detector or a proportional counter. Such measurements are very scarce. Moreover, at these energies the most dominant partial effect is the atomic photoeffect. Recently, very accurate theoretical photoelectric cross-sections are reported by Scofield[1]. Hence, it is of interest to make a systematic measurement of the total photon attenuation cross-sections at low-energies.

A small good-geometry arrangement of the Davisson Evans type with graded shields is developed in this department for use with sources of small intensity. The detector is a krypton-filled proportional counter supplied by ECIL, Hyderabad. A Co^{57} source, which emits 6·4 keV K X-rays and 14·4 keV gamma-rays, is used for the present measurements. Transmission measurements are carried out in thin foils of Cu, Mo, Au and Pb employing the usual procedures[2,3]. The cross-section is calculated using the expression

$$N = N_0 e^{-\mu t}$$

where the symbols have their usual meaning. Thin foils of different thicknesses were employed. The average values are adapted. The measured total photon attenuation cross-sections are presented in Table I. From these values the theoretical coherent and incoherent scattering cross-sections of Storm and Isreal[4], (in no case exceeding 5% of the total) are substracted. The resultant photo-electric cross sections are compared with the latest theoretical values of Scofiled[1] in the same table. The errors in the experimental values are of the order of 2%. It can be seen from Table I that there is satisfactory agreeement between theory and experiment within 3%.

The authors wish to express their gratitude to Prof. H. N. Bose, Head, Dept. of Physics, for his kind interest and constant encouragement during the course of the work.

Table I

Cross-sections (in barns/atom)

Energy (keV)		Element			
		Cu	Mo	Gold	Lead
6·4	Exp. Total:	10,430 ± 2%	48,175 ± 2%		
	Exp. Photo:	10,150 ± 2%	47,640 ± 2%		
	Th.:	10,400	47,000		
14·4	Exp. Total	..	..	61,505 ± 2%	45,251 ± 2%
	Exp. Photo			59,220 ± 2%	43,152 ± 2%
	Th.			57,801	42,403

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February 1, 1977.

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1. Scofield, J. H., *UCRL Report*, 1973.
2. Mc Grary, J. H. *et al.*, *Phys. Rev.*, 1970, **A3**, 539.
3. Premchand, K. *et al.*, *J. Phys.*, 1976, **B9**, 177.
4. Storm, E. and Isreal, H. I., *Nuclear Data*, 1970, A7.

FRANCK-CONDON FACTORS AND *r*-CENTROIDS TO HIGH QUANTUM NUMBERS FOR THE (A$^2\Sigma^+$–X$^2\Sigma^+$) TRANSITION OF AlO

In the (A–X) transition of AlO, bands with v' and v'' up to 22 have been reported[1]. Franck-Condon (FC) factors and r-centroids for this transition, using approximate Morse and less appropriate RKR potential are available[2,3], only for low vibrational quantum numbers. We are reporting here the results of our investigations on these parameters, for the above transition up to high vibrational quantum numbers *viz.*, $v' = 21$ and $v'' = 22$, using wavefunctions,

TABLE I

v' v''	FC-factors*	r-centroids (Å)
0, 0	7·296–1 (100·00)	1·645
0, 1	2·344–1 (9·4)	1·729
0, 2	3·960–1 (0·5)	1·813
1, 0	2·241–1 (17·6)	1·576
1, 1	4·441–1 (14·3)	1·660
1, 2	3·489–1 (13·8)	1·739
1, 3	7·597–2 (1·1)	1·826
2, 0	4·581–2 (0·7)	1·507
2, 1	3·047–1 (25·1)	1·585
2, 2	2·399–1 (3·9)	1·673
2, 3	3·894–1 (12·3)	1·745
2, 4	1·260–1 (1·9)	1·840
2, 5	8·421–2	1·897
3, 1	9·572–2 (4·2)	1·518
3, 2	2·962–2 (12·6)	1·600
3, 3	3·813–2	1·688
3, 4	3·801–1 (6·7)	1·765
3, 5	1·660–1 (1·0)	1·848
3, 6	8·419–3	1·910
4, 1	2·187–2	1·478
4, 2	1·379–2 (5·3)	1·527
4, 3	2·591–1 (7·1)	1·595
4, 5	3·501–1 (2·7)	1·778
4, 6	2·050 (0·2)	1·868
5, 2	1·084–3	1·489
5, 3	1·700–1 (4·3)	1·537
5, 4	2·022–1 (3·4)	1·608
5, 6	3·158–1 (1·7)	1·790
5, 7	2·393–1	1·878
6, 3	9·826–2	1·498
6, 4	1·852–1 (3·5)	1·542
6, 5	1·534–1	1·620
6, 7	2·871–1 (1·1)	1·804
7, 4	1·850–2	1·501
7, 5	1·899–1 (2·2)	1·553
7, 8	2·497–1	1·845
8, 5	8·492–2	1·510
8, 6	1·867–1 (1·0)	1·556
8, 9	2·294–1	1·856
9, 5	6·123–2	1·472
9, 6	8·977–2	1·521
9, 7	5·181–1 (0·4)	1·567
10, 9	9·148–2	1·662
10, 11	8·824–2	1·877
10, 12	8·618–2	1·925
11, 10	3·128–2	1·674
11, 12	8·642–2	1·887
11, 13	8·500–2	1·936
12, 11	1·134–2	1·686
12, 13	8·403–2	1·899
12, 14	8·394–2	1·946
13, 14	8·210–2	1·908
13, 15	8·200–2	1·855
14, 15	8·190–2	1·917
14, 16	8·100–2	1·968
15, 16	8·013–2	1·926
15, 17	9·904–3	1·978
16, 17	9·901–3	1·936
16, 18	9·800–3	1·989
17, 18	9·800–3	1·946
19, 20	9·614–3	1·965
20, 21	9·499–3	1·976
21, 22	9·313–3	1·987

* The negative numerical in each FC-factor entry is the power of 10 by which it is multiplied and the quantity in the bracket is the experimentally measured intensity.

appropriate to a more realistic RKRV potential. For this purpose the turning points based on RKRV method for the A and X states of AlO are used[4].

In the present study, four successive turning points are taken at a time and potential energy, U (r), is recorded at equal intervals of 0·01 Å for each vibrational quantum number. Ordinates of wavefunctions are determined by inserting these U (r) values in Wu's[5] expression and the corresponding FC-factors and r-centroids are computed accordingly. The results are given in Table I, along with the experimentally measured[6] intensities of the bands.

From the table it can be seen that the (0,0) is the strongest band observed and the intensities of the successive bands in the same sequence (*i.e.*, $v' - v'' = 0$) falls off rapidly as v' and v'' increase. Thus only few bands of this sequence are observed experimentally. For the neighbouring sequences, $v' - v'' = \pm 1, \pm 2, \pm 3, \cdots$, it can be seen that more bands are observed than in the (0,0) sequence, since the intensity falls off less rapidly with increasing v' and v''. All these findings are in accordance with the experimental observations.

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Dharwar 580 003, *February* 3, 1977.

1. Prasad, S. C. and Narayan, M. K., *Ind. J. Pure Appl. Phys.*, 1969, **7**, 413.
2. Tawde, N. R. and Korwar, V. M., *Proc. Phys. Soc.*, 1962, **80**, 794.
3. Sharma, A., *JQSRT*, 1967, **7**, 289.
4. Singh, N. L., *J. Sci. Res.*, (B. H. Univ.), 1969, **20** (2), 57.
5. Wu Ta You, *Proc. Phys. Soc.* (*London*), 1952, **65**, 965.
6. Hebert, G. R. and Tyte, D. C., *Proc. Phys. Soc.*, 1964, **83**, 629.

MAGNETIC PROPERTIES OF OXOVANADIUM(IV) COMPLEXES OF BIDENTATE SCHIFF BASES HAVING 'ON' DONOR ATOMS

THE VO^{2+} ion possesses one unpaired electron and the coupling of electrons in oxovandium(IV) complexes may lead to both ferromagnetic and antiferromagnetic exchange. Although spin-spin coupled oxovanadium (IV) complexes have been synthesized using dibasic tridentate Schiff bases, the monobasic bidentate, monobasic tridentate and dibasic quadridentate Schiff bases usually produce oxovanadium (IV) complexes which are magnetically dilute[1, 2]. The Cu(II) complexes of monobasic tridentate and dibasic qudridentate Schiff bases are usually involved in magnetic exchange[3, 4]. Cu(II) complexes with monobasic bidentate Schiff bases are usually magnetically dilute, although few examples of magnetically condensed Cu(II) complexes with monobasic bidentate Schiff bases have been reported[3, 4]. The difference in magnetic properties in Cu(II) and oxovanadium(IV) complexes is due to the presence of out-of-plane magnetic interaction in Cu(II) complexes and the absence of such interaction in oxovanadium(IV) complexes[2–4]. In the above light we expect that the VO^{+2} complexes of monobasic bidentate Schiff bases will not be involved in antiferromagnetic exchange. Kuge and Yamada[5] have reported that the oxovanadium(IV) complexes of I (Fig. 1) have room temperature magnetic moments in the range 1·4–1·5 B.M. indicating the presence of antiferromagnetic exchange in these complexes. An independent work of Dutta and Sengupta[6] on the oxovanadium(IV) complex of I (X = Br) indicates that the complex displays a magnetic moment of 1·75 B.M. at 297° K. We report in this communication our cryomagnetic, ESR, IR and molecular weight studies on these complexes. Our work indicates that the complexes are not involved in magnetic exchange.

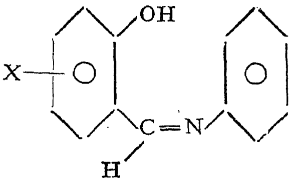
FIG. 1. I. X = Cl, Br.

The complexes were synthesized by following the method reported in the literature[6] and recrystallised from dichloromethane. *Anal.* Found: V, 10·1; N, 5·0%. Calcd. for VO (5-chloro-salicyialdehyde-aniline)$_2$, V, 9·66; N, 5·30%. Found: V, 7·9; N, 4·3%. Calcd. for VO (5-bromosalicylaldehyde-aniline)$_2$, V, 8·26; N, 4·54%. The V=O stretch of the complexes (Perkin Elmer Model 21 infrared spectrophotometer, KBr pellets) occurs in the region 984–986 cm^{-1}·. The ν (C=N) band in these complexes occurs at around 1600 cm^{-1}. The observed negative shift (~20 cm^{-1}) with respect to the free ligand indicates coordination through the nitrogen atom of the Schiff bases.

The osmometric molecular weight measurement in chloroform solutions, using a Hewlett-Packard Mechrolab Model 301 Vapour Pressure Osmometer operating at 37° C, indicate the monomeric nature of the complexes. The ESR spectra of the complexes recorded on a Varian V–4502–12 X-band spectrometer using 100 KC modulation and a 9 inch electromagnet, in dilute chloroform solutions exhibit eight line spectra (^{51}V, $I = 7/2$) with $g_{av} = 1 \cdot 98 \pm 0 \cdot 01$ and average hyperfine splittings ⟨A⟩ around 100 gauss. The ESR spectra of the complexes are typical of those of magnetically dilute oxovanadium(IV) complexes[1].

The room temperature magnetic moments of the complexes measured by the Gouy method using Hg [Co (NCS)$_4$] as the standard lie in the range 1·73–1·74 B.M. and are close to the spin-only value of 1·73 B.M. and are also comparable with the moments observed for oxovanadium(IV) complexes with other monobasic bidentate Schiff bases[1]. The magnetic moments remain almost constant in the range 83–296° K. The complexes obey the Curie-Weiss law $\chi_M^{\text{corr}} = C/T - \theta$ with Weiss constant, θ in the range 0 to + 2° K. A plot of reciprocal molar susceptbitily corrected for temperature independent paramagnetism and diamagnetism *versus* temperature is virutally linear. The complexes do not exhibit any dependence on the magnetic field strength indicating the absence of ferromagnetic exchange. We synthesized the complexes by several independent experiments and measured magnetic susceptibility separately and found deviation in the magnetic moment seldom being greater than ± 2%. In conclusion, this study indicates that the magnetic moment reported by Kug. and Yamada[5] are in error. A five coordinate square pyramidal structure is suggested to these complexes

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---

1. Selbin, J., *Chem. Rev.*, 1965, **65**, 153; *Coord. Chem. Rev.*, 1966, **1**, 293.
2. Syamal, A., *Coord. Chem. Rev.*, 1975, **16**, 309.
3. Kato, M., Jonassen, H. B. and Fanning, J. C., *Chem. Rev.*, 1964, **64**, 99.
4. Hatfield, W. E. and Whyman, R., *Transition Metal Chemistry*, 1969, **5**, 47.
5. Kuge, Y. and Yamada, S., *Bull. Chem. Soc. Japan*, 1972, **45**, 799.
6. Dutta, R. L. and Sengupta, G. P., *J. Indian Chem. Soc.*, 1971, **48**, 33.

ISOCARVOXIME

AN interesting modification of *p*-mentha-6, 8-diene-2-one oxime (carvoxime) (1) is its conversion to *p*-mentha-4 (8), 6-diene-2-one oxime (isocarvoxime) (2) through the intermediate 8-chloro-*p*-menth-6-en-2-one oxime (3).[1-3] We present here the synthesis of (2) by dehydrohalogenation of (3) with dimethyl sulfoxide-silver nitrate (DMSO-$AgNO_3$) reagent[4] and its structural evaluation by IR and PMR spectroscopy.

Upon hydrochlorination, (1) affords (3). A six proton singlet at δ 1·64 in its PMR ($CDCl_3$)* spectrum reveals the entry of Cl-atom at C–8 position. Also PMR (C_6D_6)* spectrum displays solvent shifts characteristic of α, β-unsaturated oxime moiety[5] (*vide infra*).

Reaction of (3) with DMSO-$AgNO_3$ reagent[4] furnishes homogenous (2): IR (KBr): 3420, 3200 (NOH), 1640 (C=N), 1370 $\left[>C(Me)_2 \right]$ and 770 cm⁻¹ $\left(-CH{=}C\langle \right)$ PMR ($CDCl_3$):† δ 9·50 br (1H, s, $W_{\frac{1}{2}}$ 27Hz, NOH) 6·01 (1H, sextet, J 4 and 1·5 Hz, C_6–H), 3·43 (2H, s, C_3-H), 2·94 br (2H, s, $W_{\frac{1}{2}}$ 10Hz, C_5–H), 1·88 (3 H, d, J 1·5 Hz, C_7-Me), 1·75 (3H, s, C_9–Me) and 1·69 (3H, s, C_{10}–Me); PMR (C_6D_6):† δ 9·62 br (1H, s, $W_{\frac{1}{2}}$,: 9Hz, NOH), 5·66 (1H, sextet, J 4 and 1·5 Hz, C_6–H), 3·53 (2 H, s, C_3–H), 2·68 br (2 H, s, $W_{\frac{1}{2}}$ 10 Hz, C_5–H) 1·98 (3H, s, C_7–Me), 1·63 (3H, s, C_9–Me) and 1·45 (3H, s, C_{10}–Me). These spectroscopic data are in accord with structure (2) as proposed by earlier investigators.[2,3].

8-*Chloro-p-menth-6-en-2-one oxime* (3)**:

A solution of (–)-carvoxime (1 g) in methanol (14 ml) was chilled to – 15° to – 10° and saturated with hydrogen chloride (1 hr). It was then poured on powdered ice and basified with ammonia (sp. gr. 0·89, 30 ml) and the product recrystallised from 95% ethanol (5 ml) to give the title compound (0·63 g), m.p. 135° (lit.[1] m.p. 135°); R_f 0·13; IR (KBr): 3240 (NOH), 1645 (C = N), 1385 $\left[>C(Me)_2 \right]$ and 780 (C– Cl) PMR ($CDCl_3$):* δ 6·06 br (1 H, d, J 6 Hz, C_6–H; 3·48 (1H, q, J 14 and 2 Hz, C_{3eq}–H), 2·08 (1H, obscured t, J 14 Hz, C_{3ax}–H), 1·88 (3 H, s, C_7–Me) and 1·64 (6H, s, C_9– and C_{10}–Me); PMR (C_6D_6)*: δ 9·80 (1H, s, NOH), 5·60 (1H, d, J 6Hz, C_6–H), 3·56 (1H, q, J 14 and 2Hz, C_{3eq}–H), 1·84 (1 H, obscured t, J 14Hz, C_{3ax}–H), 1·96 (3H, s, C_7–Me) and 1·24 (6 H, s, C_9– and C_{10}–Me).

Isocarvoxime (2):

To (3) (2 g) dissolved in DMSO (20 ml) was added a solution of $AgNO_3$ (1·7 g) in DMSO (10 ml) and the mixture stirred for an hour. Usual work up afforded a sticky mass which when triturated with petroleum ether (b.p. 40–60°, 10 ml) gave (2) (0·15 g), m.p. 142–143° (from petroleum ether) (lit.[1] m.p. 143°); R_f 0·28; IR (KBr), PMR ($CDCl_3$) and PMR (C_6D_6) as described above.

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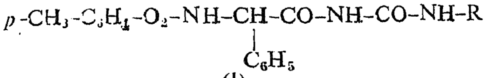
November 17, 1976.

Symbols used.—* 100 MHz, † 90 MHz, ** For general directions see reference 6.

1. Goldschmidt, H. and Kisser, E., *Chem. Ber.*, 1887, **20**, 2073.
2. Baeyer, A., *Ibid.*, 1896, **29**, 12.
3. Wallach, O., *Annalen*, 1906, **346**, 277.
4. (*a*) Mehta, G., *Indian J. Chem.*, 1971, **9**, 559.
   (*b*) Bakthan Singaram and James Verghese, *Ibid.*, 1976, **14 B**, 479.
5. Wolkowski, Z. W., Thoai, N. and Wiemann, J., *Tetrahedron Letters*, 1970, 93.
6. Saraswathi, G. N., Muraleedharan, N. V. and Verghese, J., *Indian J. Chem.*, 1974, **12**, 561.

PREPARATION OF D (–)-α-*p*-TOLUENESULPHONAMIDOPHENACETYL ARYLUREAS

UREA derivatives possess wide therapeutic activity. Phenylacetamido ureas are active anticonvulsants and used for different types of epilepsy[1-2]. Sulphonamides have been reported to be biologically versatile compounds for diseases like cancer[3], tuberculosis[4], diabetes[5], malaria[6], leprosy and as anticonvulsant[8]. We have prepared D (–)-α-*p*-toluene sulphonamidophenylacetyl arylureas of type (I) by condensing *p*-toluenesulphonamidophenylacetic acid (as acid chloride) with different phenyl ureas. D (–)-*p*-Toluenesulphonamidophenylacetic acid was prepared by reacting D (–)-α-amino-phenylacetic acid $[\alpha]_D^{20}$ —157·8 (1 N HCl), with *p*-toluene-sulphonyl chloride. The optical rotations were taken in dioxan.



$$p\text{-}CH_3\text{-}C_6H_4\text{-}O_2\text{-}NH\text{-}\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\text{-}CO\text{-}NH\text{-}CO\text{-}NH\text{-}R$$

(I)

R = Aryl

EXPERIMENTAL

1. *Preparation of D (—)-α-p-toluenesulphonamidophenylacetyl chloride*:

D (—)-α-p-toluenesulphonamidophenylacetic acid was prepared by condensing D (—)-α-aminophenylacetic acid with p-toluene sulphonyl chloride[9] % yield 66, m.p. 120–22°, $[\alpha]_D^{30}$ −101 (c, 0·5, 1, 1 in dioxane). The acid was then converted to acid chloride by the action of PCl_5[10], % yield 72, m.p. 155–57°, $[\alpha]_D^{30}$ −88 (c, 1, 1, 1 in dioxan). It gave correct N analysis.

2. *Preparation of D (—)-α-p-toluenesulphonamido-phenylacetyl arylureas*:

A mixture of D (—)-α-p-toluenesulphonamidophenylacetyl chloride (0·002 mole), different phenyl ureas (0·002 mole) and benzene (15 ml.) was heated under reflux at 60–70° C. for 4 hours. The product was isolated after removal of solvent and crystallised from alcohol. The substituent R, yield in %, m.p. and optical rotation (c, 1, 1, 1 in dioxan) are as under. Phenyl, 92, 90–92°, −71; *o*-Tolyl, 89, 138–40°, −68; *m*-Tolyl 88, 82–84°, −55; *p*-Tolyl 93, 105–07°, −72; *o*-Anisyl 82, 85–87°, −76; *p*-Anisyl 70, 87–89°, −56; *o*-Chlorophenyl 88, 80–82°, −75; *m*-Chlorophenyl 91, 104–06°, −62; *p*-Chlorophenyl 93, 106–08°, −81; α-Naphthyl 84, 70–72°, −61. All compounds gave correct N analysis.

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Department of Chemistry, V. N. KHUNT.
Saurashtra University, A. R. PARIKH.
Bhavnagar, *December* 13, 1976.

---

1. Spiceman, M. A., Ceiszler, A. O. and Clase, W. J., *J. Am. Chem. Soc.*, 1948, 70, 4189.
2. Mishra, V. S. and Prakash, S., *J. Ind. Chem. Soc.*, 1974, **51**, 969.
3. Clayson, D. B., Pringle, J. A. S. and Ronser, G. M., *Biochem. Pharmacol.*, 1967, **16** (4), 614.
4. Bossche, W. V. D., *Pharm. Tijdschr. Belg.*, 1965, **42** (8), 156; *Chem. Abstr.*, 1966, **64**, 6408*h*.
5. Horstamann, H., Pluemple, H. and Schroeder, *S. African Patent*, 1969, 6904136; *Chem. Abstr.*, 1970, **73**, 25497*n*.
6. Beeslay, W. N., Peters, W. and Mangen, K., *Ann. Trop. Med. Parasitol.*, 1968, 62 (3), 288.
7. Tarbini, G., *Inst. Congr. Chemother. Proc.*, 1967, **5th 2** (2), 909.
8. Goores, E. and Lange, B., *Acta Biol. Med.*, 1968, **21** (2), 193.
9. Vogel, A. I., *A Textbook of Practical Organic Chemistry*, p. 437.
10. Beechamp, A. R., *J. Am. Chem. Soc.*, 1957, **79**, 3257.

FLAVONE FROM *ARTEMISIA HERBA-ALBA* A SYNTHETIC STUDY

A NEW flavone isolated from *Artemisia herba-alba* was assigned its constitution by Segal *et al.*[1] as 5, 4′-dihydroxy-6, 7, 3′-trimethoxyflavone had m.p. 147° whereas Morita *et al.*[3], who isolated its 4′-monoglucoside from *Cirsium lineare*, reported that 5, 4′-dihydroxy-6, 7, 3′-trimethoxyflavone had m.p. 208–10°. However, Krishnamurti *et al.*[2] made this compound in some other connection and recorded its m.p. 200–01°. In view of the different melting points reported for the three different samples of the same flavone, its synthesis by another method was considered desirable to settle this discrepancy. For this purpose, 5, 6, 7, 3′-tetramethoxy-4′-benzyloxyflavone obtained by selenium dioxide oxidation of 2′-hydroxy-3, 4′, 5′, 6′-tetramethoxy-4-benzyloxychalkone[4] when subjected to the catalytic debenzylation and then subsequent selective demethylation yielded 5, 4′-dihydroxy-6, 7, 3′-trimethoxyflavone. This dihydroxytrimethoxyflavone was identical with the sample made by the procedure described by Krishnamurti *et al.*[2].

The natural sample kindly provided by Dr. R. Segal was insufficient for the melting point or mixed melting point determinations which could have settled this discrepancy. Hence the constitution assigned to the flavone isolated[1] from *A. herba-alba* needs revision.

Experimental

5, 6, 7, 3′-Tetramethoxy-4′-benzyloxyflavone.—A mixture of 2′-hydroxy-3, 4′, 5′, 6′-tetramethoxy-4-benzyloxy chalkone[4] (1·0 g), selenium dioxide (1·7 g), and amyl alcohol (40 ml) was refluxed for 24 hrs and then worked out. The flavone crystallised from benzene-acetone as colourless needles, m.p. 178–79°. Mixed melting point with the sample made by the other method* was unchanged.

5, 6, 7, 3′-Tetramethoxy-4′-hydroxyflavone.—The above benzyloxytetramethoxyflavone (0·5 g) in ethyl acetate (50 ml) and Pd–C (0·3 g) were stirred in an atmosphere of hydrogen till debenzylation completed. 5, 6, 7, 3′-Tetramethoxy-4′-hydroxyflavone crystallised from benzene as colourless needles, m.p. 208–10° (Found: C, 63·3; H, 4·8. $C_{19}H_{18}O_7$ requires: C, 63·68; H, 5·02%).

5, 4′-Dihydroxy-6, 7, 3′-trimethoxyflavone.—The above monohydroxytetramethoxyflavone (0·3 g) in acetonitrile (5 ml) and aluminium chloride (0·3 g) were refluxed for 4 hrs., and then worked out. The demethylation product crystallised from alcohol as pale yellow needles, m.p. 200–201°. Mixed melting point with the sample obtained by the other method[2] was unchanged.

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* Krishnamurti *et al.*[2] had reported the m.p. 144–46°. However this flavone, when made by their method, was found to have m.p. 178–79°.

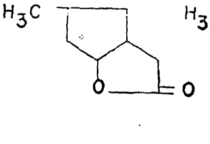
1. Segal, R., Cohen, D., Sokoloff, S. and Zaitschek, D. V., *Lloydia*, 1973, 36, 103.
2. Krishnamurti, M., Seshadri, T. R. and Sharma, N. D., *Indian J. Chem.*, 1973, 11, 201.
3. Morita, N., Shimizu, M. and Arisawa, M., *Phytochemistry*, 1973, 12, 421.
4. Horhammer, L., Wagner, H., Graf, E. and Farkas, L., *Ber. dt. chem. Ges.*, 1965, 98, 548.

FRIEDEL-CRAFTS ALKYLATION OF AROMATIC HYDROCARBONS WITH SUBSTITUTED ALICYCLIC LACTONES

THE aluminium chloride-catalysed condensation of aromatic hydrocarbons with simple substituted alicyclic lactones like the lactone of 4-methyl-2-hydroxycyclohexaneacetic acid was found by Chatterjee and Bhattacharya[1] to afford a mixture of secondary alkylates consisting of 2-aryl and 3-aryl-4-methylcyclohexaneacetic acid to the exclusion of the tertiary alkylate, *e.g.*, the 4-aryl isomer. The facile rearrangement in the cyclopentane system has also been observed by Chatterjee and coworkers[2] who studied the catalysed alkylation of aromatic hydrocarbons with the lactone of cyclopentanol-2-acetic acid. We now report the synthesis of lactone of 4-methyl-2-hydroxycyclopentaneacetic acid (I) and the Lewis acid catalysed alkylation of aromatics with it.

The lactone (I) was prepared from β-methyl adipic acid, m.p. 93° obtained from 4-methylcyclohexanol by oxidation with 50% nitric acid in the presence of V_2O_5. The diethyl ester, b.p. 130–32°/14 mm, on Dieckmann cyclisation furnished 4-methyl-2-carbethoxycyclopentanone, b.p. 90–92°/1 mm., which on alkylation by ethyl chloroacetate in the presence of sodium ethoxide afforded ethyl 4-methyl-2-carbethoxy-2-acetate, b.p. 132–35°/0·5 mm. This on hydrolysis by 1:1 HCl yielded 4-methylcyclopentane-2-one-1-acetic acid, b.p. 155–60°/2 mm., semicarbazone, m.p. 210°, which on $NaBH_4$ reduction gave a stereoisomeric mixture of the desired lactone (I), b.p. 95–100°/1 mm n_D^{30} 1·4650, containing a major amount of the trans variety[3].

The Friedel-Crafts condensation of the lactone (I) with benzene in the presence of $AlCl_3$ gave as expected a secondary alkylate consisting of a stereoisomeric mixture of 4-methyl-2-phenylcyclopentaneacetic acids (II), b.p. 155–57°/0·6 mm as the exclusive product, isolated through the ethyl ester, b.p. 140–41°/0·6 mm in 78% yield together with a small amount of 3 methylcyclopentaneacetic acid formed by hydride transfer and consequent reduction of the lactone (I). The acid (II) on PPA cyclisation afforded 2-methyl-6-keto 6, 7, 8, 9-tetrahydro-4, 5-benzindane (III), b.p. 137–38°/1 mm. semicarbazone m.p. 206°. DNP derivative m.p. 209° as a stereoisomeric mixture. The structure of the ketone was confirmed by its reduction and dehydrogenation to 2-methyl-4, 5-benzindane, picrate m.p. 91° TNB complex m.p. 103° as well as by its unequivocal synthesis starting from ethyl 4-methylcyclopentane-2-one-1-acetate and phenyl lithium followed by Paar reduction of the resulting lactone and cyclisation.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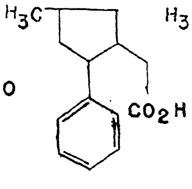
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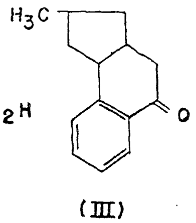
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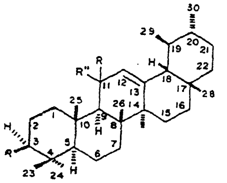
1. Chatterjee, D. N. and Bhattacharya, S. P., *Tetrahedron*, 1971, 27, 4153.
2. —, — and Sarkar, M., *Curr. Sci.*, 1977, 46, 74.
3. House, H. O., Babad, H., Toothill, R. B. and Noltes, A. W., *J. Org. Chem.*, 1962, 27, 4141.

ISOLATION OF α-AMYRIN STEARATE, β-AMYRIN AND LUPEOL PALMITATES FROM THE COSTUS LEAVES

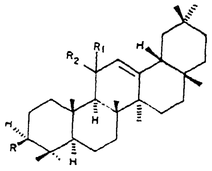
THE isolation of taraxasterol and its acetate from the hexane extract of the leaves (and stalks) of the costus plant, *Saussurea Lappa* clarke, has been reported.[1] By chromatography of the hexane extract over alumina and silver nitrate impregnated silicic acid, followed by cyrstallisation of some of the fractions, three low melting esters A, B, C have been isolated in the pure state. These have been identified as α-amyrin stearate, β-amyrin palmitate and lupeol palmitate respectively, by chemical reactions and comparison of physical constants and spectral data with those reported in literature.[2a,b,c] Ester A, $C_{48}H_{84}O_2$ m.p. 46–48° (EtOH + Me_2CO; 1:4) $(\alpha)_D$ +61° (benzene, c, 1·84). On saponification, yields stearic acid, $C_{18}H_{36}O_2$, M^+ 284, m.p. 69° (EtOH) and α-amyrin (I), $C_{30}H_{50}O$, M^+ 426, m.p. 181–183° (hexane + 10%

benzene), $(\alpha)_D$ + 83° ($CHCl_3$, c, 1). The latter is converted for characterisation into the acetate (II)[3], $C_{32}H_{52}O_2$, m.p. 218–220° (EtAc), $(\alpha)_D$ + 81° ($CHCl_3$, c, 1) and also to 11-keto acetate[4] (III), $C_{32}H_{50}O_3$, m.p. 274°–276° (EtAc), $(\alpha)_D$ + 97° ($CHCl_3$, c, 0·6) by sodiumdichromate[5] and acetic acid oxidation of (II).

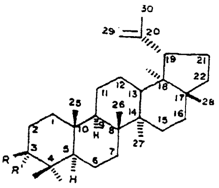
Ester B, $C_{46}H_{80}O_2$, M^+ 664 m.p. 76–78° (Me_2CO + 10% hexane), $(\alpha)_D$ + 57° (benzene, c, 2·8) gives on saponification palmitic acid $C_{16}H_{32}O_2$, M^+ 256, m.p. 62° (EtOH) and β-amyrin (IV) $C_{30}H_{50}O$, M^+ 426, m.p. 193–194° (hexane + 10% benzene), $(\alpha)_D$ + 91° (benzene; c, 2). The latter is converted for characterisation into the acetate (V), $C_{32}H_{52}O_2$, m.p. 236–238° (EtAc), $(\alpha)_D$ + 82° ($CHCl_3$, c, 1·5) and also to 11-keto acetate (VI), $C_{32}H_{50}O_3$, m.p. 264° (EtAc), $(\alpha)_D$ + 110° ($CHCl_3$, c, 0·9) by oxidation of (V) with sodium dichromate and acetic acid[5].



R = OH ; R' = R'' = H
II R = $OCOCH_3$, R' = R'' = H
III R = $OCOCH_3$ R', R'' = O
Ester A, R = $OCOC_{18}H_{36}$; R' = R'' = H



IV R = OH, $R_1 = R_2$ = H
V R = $OCOCH_3$; $R_1 = R_2$ = H
VI R = $OCOCH_3$; R_1, R_2 = O
VII R = $OCOC_{16}H_{32}$; R_1, R_2 = O
Ester B, R = $OCOC_{16}H_{32}$, $R_1 = R_2$ = H



VIII R = OH, R' = H
IX R = $OCOCH_3$, R = H
X R, R' = O
Ester C, R = $OCOC_{16}H_{32}$, R' = H

Oxidation of ester B with sodiumdichromate and acetic acid furnishes the 11-keto β-amyrin palmitate (VII), $C_{46}H_{78}O_3$, M^+ 678, m.p. 148° (EtAc). It shows IR bands at 1736 (>C=O of ester), 1660 (>C=O conj. ketone) and 1613 cm^{-1} (>C=C< conj); $UV\lambda_{max}$ 248 mμ, ϵ_{max} 12,500; NMR (CCl_4) signals at τ: 7·75 (1H, C_9-proton adjacent to C=O group); 5·53 C_3-proton) and at 4·5 (1H, olefinic proton at C_{12}).

Ester C, $C_{46}H_{80}O_2$, m.p. 77–78° (Me_2CO), $(\alpha)_D$ + 26° (benzene, c, 2·8), on saponification gives palmitic acid and lupeol (VIII), $C_{30}H_{50}O$, M^+ 426, m.p. 214–216° (MeOH + Me_2CO; 2:1), $(\alpha)_D$ + 28° ($CHCl_3$, c, 2·5). The latter is converted for characterisation into the acetate (IX) $C_{32}H_{52}O_2$, m.p. 215–217° (EtAc), $(\alpha)_D$ + 43° ($CHCl_3$, c, 1·2) and also to lupenone (X), $C_{30}H_{48}O$, H^+ 424, m.p. 169–171° (Me_2CO), $(\alpha)_D$ + 57° ($CHCl_3$, c, 1·5); 2·4 DNP ($C_{36}H_{52}N_4O_4$, m.p. 212–214°, by oxidation with Jones' chromic acid reage

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---

1. Namboodiripad, C. P., Kulkarni, G. H. an Kelkar, G. R., *Curr. Sci.*, 1968, **37**, 550.
2. (*a*) Simonsen, J. and Ross, W. C. J., *The Terpene* Cambridge University Press, 1957, **4**, p. 116.
   (*b*) — and —, *Ibid.*, 1957, **4**, p. 176.
   (*c*) — and —, *Ibid.*, 1957, **4**, p. 331.
3. V. K. Hinge's thesis *Terpenoids*, Poona Universit 1966, pp. 24, 25 and 26.
4. Yagishita, K. and Nashimura, M., *Agri. and Bi Chem.*, (*Japan*), 1961, **25**, 517 and 844.
5. Kulkarni, G. H., *Chem. and Indy.*, 1970, p. 1498.

ZINC (II) COMPLEXES WITH 2-ACETYL PYRIDINE

DIVALENT $Zinc^{2+}$ has a completely filled $3d^{10}$ non-bonding shell and hence usually forms four-coordinated tetrahedral compounds utilising $4S4P^3$ hybrid orbitals for bonding. Several complexes of Zinc (II) with substituted pyridines have been reported[1]. Similar complexes of Zinc (II) with 2-acetyl pyridine with different anions have been isolated and characterised now.

An ethanolic solution of the respective Zinc salts were treated with an ethanolic solution of 2-acetyl pyridine in 1 : 2 proportion. In all the cases the crystalline compounds separated after prolonged stirring. The compounds were filtered, washed with ethanol and dried *in vacuo*. The purity of the compounds was established by chemical analysis. The conductance measurements were made in acetone solution using a dip type cell. Infrared spectra were recorded using Unicam SP–200 Spectrophotometer on Nujol mulls. The relevant data are recorded in Table I.

All the complexes are diamagnetic as expected for a closed shell d^{10} configuration of the metal ion. The low conductance in acetone medium for the bromo and cyanato complexes are indicative of the non-electrolyte nature. The high conductance values for the perchlorato and thiocyanato complexes are indicative of 1:2 electrolytes. The I.R. spectrum in the region 5000–650 cm^{-1} were studied. A broad and strong band at 1020–1110 cm^{-1} for the perchlorate complex and a band at 2070 cm^{-1} for the thiocyanate complex confirmed the presence of ionic perchlorate[5,6] and ionic thiocyanate[7] respectively. The bands at 1380 cm^{-1} suggest[8] a N-bonded cyanate group. So, the complexes reported in the present communication can be assigned the following formulae $[Zn L_2 Br_2]$, $[Zn L_2 (NCO)_2]$, $[Zn L_4](CNS)_2$ and $[Zn L_4] (ClO_4)_2$ (where L = 2-acetyl-pyridine). They can be presumed to be four co-ordinated, tetrahedral complexes similar to those reported earlier[1,9,10].

TABLE I

Analysis, M.P. and conductance of the zinc (II) complexes with 25 acetyl pyridine

Formula	M.P. °C	Conductance in mhos	% Metal Found	% Metal Calculated	% halogen sulfur Found	% halogen sulfur Calculated
$[ZnL_4](ClO_4)_2$	210	250	8·75	8·70		
$[ZnL_4](SCN)_2$	138	166	9·70	9·80	(S) 9·4	9·6
$[ZnL_2(NCO)_2]$	190	35	16·01	16·6		
$[ZnL_2Br_2]$	185	6	13·65	13·99	(h) 33·58	34·2

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1. Guru, S. and Ramana Rao, D. V., *Curr. Sci.*, 1967, 36, 317.
2. — and —, *Z. Anorg. Chem.*, 1968, p. 358.
3. Mohapatra, B. K. and Ramana Rao, D. V., *Ibid.*, 1970, p. 372.
4. — and —, *Inst. of Chemists* (*India*), 1970, **5**, 199.
5. Nakamoto, K., *Infrared Spectra of Inorganic and Cordination Compounds*, John Wiley and Sons, Ed., 1963, 107.
6. Patel, R. N. and Ramana Rao, D. V., *Curr. Sci.*, 1967, 36, 262.
7. Forster, D. and Goodgamme, D. M. L., *J. Chem. Soc.*, 1965, p. 1287.
8. Sahai, R. B. N., Chaturbedi, A. P. and Mishra, L. K., *Ind. J. Chem.*, 1976, **14**, 360.
9. Mohapatra, B. K. and Ramana Rao, D. V., *Ibid.*, 1970, 8, 564.
10. Allan, J. R., Brown, D. H., Nuttal, R. H. and Sharp, D. W. A., *J. Chem. Soc.*, 1966, p. 1031.

STUDIES ON CHOLINESTERASE ACTIVITY IN THE PLASMA, RBC AND BRAIN OF STREPTOZOTOCIN TREATED RATS

ACETYLCHOLINESTERASE (acetylcholine hydrase, EC 3.1.1.7) is present in many animal tissues and besides functioning as a neural transmitter it also aids in the mechanism of cell permeability and osmotic fragility, specially in the red blood cells (RBC)[1,2]. A decreased cholinesterase (ChE) activity was noted in the liver and blood of rats maintained on an atherogenic diet[3] and a similar thing was reported in undernourished states[4]. An increase of serum ChE activity was noted in albino rats[5] and dogs[6] in which diabetes was induced either by alloxan treatment or by pancreatectomy. The present communication reports observations made during studies conducted to determine the variations in ChE levels in rats in which diabetes was induced by the administration of streptozotocin·

The test rats (male albino rats of the Wistar strain) were injected intravenously streptozotocin in citrate buffer (ph 4·5), at a dose of 60 mg/kg body weight, while the control rats received only injections of the buffer. The test rats were given 2% glucose during the first 48 hours of injection. After this, both test rats and control rats were given food and water *ad libitum*. The rats were sacrificed four weeks after the injection. Plasma and RBC were separated by centrifugation at 2000 RPM in a refrigerated centrifuge. The packed RBC were washed twice with 0·9% saline and finally resuspended in the same solution. The brain samples were homogenized in distilled water to a final concentration of 10%. AcetylChE and butyrylChE were assayed in plasma, RBC and brain by the method of Hestrin[8]. Protein was estimated by the method of Lowry *et al*[9] and blood sugar by the method of Nelson and Somogyi[10].

Tables I and II summarize the results obtainede It was noted that (compared to the controls) the activities of both ChE were elevated in plasma as well as RBC of the test rats. No significant differences were. noted between the test and control rats in any of the ChE in brain samples. The increase of blood ChE activity in pancreatectomized dogs was attributed by Fokin[6] as due to impaired liver function and this was confirmed by Mc Daniel *et al.*[11] who noted that alloxan diabetic rats who showed impaired conversion of L-tryptophan to niacin showed this conversion after treatment with insulin. The toxic effects of strepto-

TABLE I

Animal weights, tissue weights and blood sugar in normal and streptozotocin diabetic rats

(Values are averages of six rats and standard deviations)

	Normal rats	Streptozotocin diabetic rats
Rat (g)	247·12	190±13
Brain (g)	1·46±0·21	1·32±0·34
Liver (g)	6·32±0·73	4·98±0·70
Kidney (g)	1·53±0·28	1·21±0·35
Blood sugar (mg %)	72±8	298±30

TABLE II

Cholinesterase activity in plasma and RBC of control and streptozotocin-treated rats

Values are averages of six rats and standard deviations

Enzyme activity*		Control rats	Test rats
Plasma	AchE	$44 \cdot 8 \pm 7 \cdot 4$	$57 \cdot 6 \pm 6 \cdot 5'$
	BChE	$23 \cdot 1 \pm 4 \cdot 1$	$32 \cdot 5 \pm 7 \cdot 0'$
RBC	AChE	452 ± 21	$558 \pm 28''$
	BChE	$22 \cdot 8 \pm 7 \cdot 2$	$38 \cdot 4 \pm 9 \cdot 8''$

* Expressed as micromoles of substrate utilized/ml-hour.

AChE, acetylcholinesterase; BChE,-Butyrylcholinesterrase; $'p < 0 \cdot 05$ and $''p < 0 \cdot 01$—Student's '*t*' test.

zotocin were noted to last only for 48 to 72 hrs after the injection, which indicated that a different mechanism might be operating to account for elevated plasma and RBC ChE activities in the case of these rats unlike the alloxan diabetic rats. It is interesting to note that Keply[13] reported alteration in ChE activity of RBC of human subjects with protein caloric malnutrition. In order to check whether any such mechanism is operative in streptozotocin-treated rats, further studies are in progress.

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---

1. Greig, M. E. adn Holland, W. C., *Arch. Biochem.*, 1949, **23**, 370.
2. Milstoc, M. and Wolfson, A. H., *Am. J. Clin Path.*, 1972, **57**, 494.
3. Aptekar, S. G. and Il'inich, A. A., *Vopr. Pitaniya*, 1964, **23** (6), 121 (*C.A.*, 1965, **62**, 2037 g).
4. Miholjicic, M., Salamon, T. and Nikalin, A., *Enzymologia*, 1964, **27** (2), 81.
5. Fokina, A. A., *Byul. Eksperim. Biol. i. Med.*, 1964, **58** (10), 41, (*C.A.*, 1965, 62, 8232 g).
6. —, *Pathol. Firiol. Eksperim. Terapiya*, 1965, **9** (2), 75 (*C.A.*, 1965, 63, 8860 f).
7. Junod, A., Lambert, A. E., Steuffaoher, W. and Renold, A. E., *J. Clin. Invest.*, 1969, **48**, 2129.
8. Hestrin, S., *J. Biol. Chem.*, 1949, **180**, 249.
9. Lowry, O. H., Roseboreugh, N. J., Farr, A. L. and Randall, R. J., *Ibid.* 1951, **193**, 265.
10. Nelson and Somogyi, *Howks Practical Physiological Chemistry*, McGraw-Hill Book Company, Inc., 1954, p. 573.
11. Mc Daniel, E. G., Hundley, J. M. and Sebrell, W. H., *J. Nutrn.*, 1956, **59**, 407.
12. Junod, A., Lamert, A. E., Orci, L., Picket, R., Gonet, A. E. and Renold, A. E., *Proc. Soc. Exp. Biol. Med.*, 1967, **126**, 201.
13. Kaply, S. S., *Ind. J. Biochem. Biophys.*, 1975, **12** (3), 284.

KNUDSEN DIFFUSION AND NEGATIVE ACTIVATION ENERGY OF ADSORPTION

NEGATIVE activation energy in chemisorption of gases on solids has been observed by many workers like Sastri and Ramanathan[1], Taylor and Liang[2], Kubokawa and Taylor[3], Low and Taylor[4] and by Decrue and Susz[5]. In most cases, surface heterogeneity effects have been proposed to account for this. In our case we observed also the occurrence of negative activation energy in the temperature range 31°–253° C for oxygen adsorption on copper vanadate pretreated with hydrogen at 40 mm pressure and 130° C and we propose a Knudsen diffusion model to account for this behaviour.

In 1953, Sutherland and Winfield[6] have developed a mathematical model for Knudsen diffusion for rapid gas adsorption on porous solids and in 1965, Peers[7] has shown that such a model gives rise to an Elovich rate equation in a constant volume system. However no further work has been carried out in this aspect.

The conditions for the Knudsen diffusion being the rate-controlling step (according to these authors) are:

1. Pressure should be less than 2 atm.
2. Pore radius should be < 100 Å.
3. Rapid uptake of gas.
4. Rapid establishment of equilibrium between the pore wall and the gas molecules.
5. Adsorption taking place in a constant volume system.

For such conditions, Sutherland and Winfield[6] have deduced

$$-\frac{dp}{dt} = \frac{p_0 Y}{\pi^{1/2} t} \qquad (1)$$

where,

p_0 = initial pressure,

$$Y = \frac{D_k \pi b r^2 t^{1/2}}{V D^{1/2}} \qquad (2)$$

b — No. of cylindrical pores, each of length 1,
V = Volume of system,
r = Average pore radius,
D_k = Knudsen diffusion constant

$$\approx 1 \cdot 33\, r \left(\frac{2RT}{\pi M}\right)^{1/2}$$

$$D_k / D^{1/2} = 1 \cdot 94\, k_e^{1/2} (RT)^{3/4} (\pi M)^{-1/4} \qquad (3)$$

k_e = Intrinsic adsorption coeff.

Now,

$$\frac{d \ln K_p}{dT} = \frac{\Delta H}{RT^2}$$

Let $\Delta H = -Q$ where Q is the heat of adsorption (it is negative since the adsorption is exothermic).

So,

$$\frac{d \ln k_e}{dT} = \frac{-Q}{RT^2}$$

[illegible]

Fig. 1

In [illegible] values [illegible] described as [illegible] increases as temperature decreases. But at higher temperatures [illegible] the slope of the 2nd stage [illegible] Moreover, the break [illegible] appears in the region

[illegible] Elovich plots show deviation [illegible] in slope is evidently due to [illegible] factors remaining constant. The [illegible] at the 2nd stage (Q_2) is not only [illegible] Q_1 [illegible] higher) but also Q_2 [illegible] RT [illegible] has little influence in changing [illegible] 2nd stage at different temperatures. Hence [illegible] may be used to test whether Knudsen diffusion is rate controlling or not, and [illegible] of the sample is known, this may be [illegible] method for the evaluation of heat [illegible] Q from the slope of dp/dt vs. $1/p^{1/2}$ [illegible]

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1. [illegible] M. V. C. and Ramanathan, K. V., *J. Phys. Chem.*, [illegible], 86, 220.
2. [illegible], H. S. and Liang, S. C., *J. Am. Chem. Soc.*, 1947, 69, 1306.
3. K[illegible], S. Y. *et al.*, *J. Phys. Chem.*, 1956, 60, [illegible]
4. L[illegible], M. J. D. and Taylor, H. A., *J. Electrochem. Soc.*, 1959, 106, [illegible]
5. [illegible], J. and Susz, B., *Helv. Chim. Acta*, 1956, 39, [illegible]
6. Sutherland, K. L. and Winfield, M. E., *Austral. J. Chem.*, 1953, 6, 234, 244.
7. P[illegible], A. M., *J. Catal.*, 1965, 9, [illegible]
8. Bhattacharyya, S. K. and Datta, N. C., *Kinetika i Kataliz*, 1975, 16 (1), 221.

CYCLIC CHANGES IN PERIPHERAL PLASMA PROGESTERONE IN BUFFALO HEIFERS

In cows with silent heat Dobrowolski and coworkers[1] observed significantly lower levels of blood progesterone at all stages of the oestrous cycle. No such study has been reported in buffalo. The investigation was undertaken to determine the peripheral plasma progesterone levels in buffalo heifers during the various periods of oestrous cycle as incidence of silent heat in this species is quite high during summer[2-4].

The study was carried out during June-July, 1976 with 9 Murrah buffalo heifers 3-4 years of age and average weight [illegible] kg. The animals were checked [illegible] daily at 8 A.M. and 4 P.M. using vasectomized bull. Jugular venous blood was collected in heparinized tubes, which were immediately centrifuged and plasma stored at [illegible] 15° C. Progesterone was determined by the competitive protein binding assay as described by Murphy[5]. The efficiency value of the method was 85%.

All the buffalo heifers were in 'silent' heat with no apparent symptoms of oestrus. On rectal palpation of the genitalia, mucous discharge invariably came out. Corpus luteum (CL) could only be detected in 6 animals at 10th and 15th day of the service; 5 heifers repeated heat with a cycle length of 23 ± 1·5 days and one heifer became pregnant.

The average plasma progesterone levels (ng/ml) of the 5 cycling buffalo heifers on day 0, 3, 5, 10, 15 and 21 of the oestrous cycle (day 0 = day of heat) were $< 0\cdot10$, 0·11, 0·10, 0·24, 0·24 and 0·17 respectively. The progesterone levels in one heifer which conceived were 0·22, 0·36, 0·60, 0·84, 1·96 and 2·54 ng/ml. The 3 heifers in which heat was non-ovulatory the values were $< 0\cdot1$ ng/ml at all intervals.

In cows, the average plasma progesterone concentration (ng/ml) during the oestrous cycle has been reported to be lowest at oestrus (0·2 to 2·0) which remain low for a period of 2 to 4 days, increase to maximum values (5·9 to 9·0) at about day 14 and again register a decline during the pre-oestrus period to nadir values of $< 1\cdot5$[1,6–8]. It is obvious that in buffalo heifers much lower values of plasma progesterone could be obtained at all stages of the cycle as compared to cow, although its temporal pattern remain similar in the two species. During early pregnancy in cows, upto first 14 days the mean levels of plasma progesterone remained similar to normal cycles, which then declined in cyclic animals but not in those which became pregnant. In the buffalo heifer which conceived, higher levels of progesterone were obtained at all stages of the oestrous cycle.

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---

1. Dobrowolski, W., Snochowski, M. and Staszkiewicz, M., *Polskie Archiwum Weterynaryjne*, 1973, **16**, 671 (cited from *Anim. Breed. Abstr.*, **43**, 1060).
2. Mac Gregor, R., *Vet. Rec.*, 1941, **53**, 443.
3. Hafez, E. S. E., *J. agric. Sci.* (*Camb.*), 1954, **44**, 165.
4. Luktuke, S. N. and Roy, D. J., *Indian J. Vet. Sci.*, 1964, **34**, 160.
5. Murphy, B. E. P., *J. Clin. Endocr. Metab.*, 1967, **27**, 973.
6. Pope, G. S., Gupta, S. H. and Munro, I. B., *J. Reprod. Fert.*, 1969, **20**, 369.
7. Domeki, I., Nakahara, T. and Yamauchi, N., *Japanese J. Anim. Reprod.*, 1974, **20**, 71.
8. Donaldson, L. E., Bassett, J. M. and Thorburn, G. D., *J. Endocr.*, 1970, **48**, 599.

AGE OF KALPATTA GRANITE, KERALA STATE

The Kalpatta granite, a medium to coarse-grained biotite granite, occurs among the Pre-cambrian crystalline rocks in the Calicut District, Kerala State. Studies on the granite and the associated country rocks in and around Kalpatta (11° 36′ 15″: 76° 5′ 5″), located on the south-western margin of the granite body, throw light on its age and relation with the associated biotite hornblende gneisses.

A penetrative megascopic foliation (S_I) is characteristic of both the granite as well as the associated gneisses. While S_I in the gneisses is defined by planar preferential alignment of biotite flakes and hornblende grains, in the granite it is after biotite flakes. S_I foliation is axial surface type with respect to the earliest recognisable folds (F) in the gneisses. The appearance of S_I in these rocks is a significant deformational event indicative of the accompanying metamorphic peak and can therefore be used as a time-marker for establishing the age relation between the granite and the gneisses.

Charnockites and granulites occurring as occasional lenses in the gneisses carry imprints of polymetamorphism[1]. Grains having a pyroxene core with a hornblende or biotite rim are common in these lenses. Obviously a lower grade amphibolite facies metamorphism has been printed on an earlier granulite facies assemblage. Since the penetrative axial surface foliation (S_I) is after biotite and hornblende, its development marks the peak of the retrograde amphibolite facies metamorphism in time.

The granite is traversed by pegmatitic and aplitic veins of two age groups. The early aplite veins bear evidences of metamorphic recrystallisation as they have developed S_I foliation after biotite. S_I traces in the granite run uninterruptedly through these aplites, cutting across vein boundaries. The early pegmatite veins, though free from megascopic foliation, have been strongly boudinaged and in some cases the pegmatitic material has even flowed parallel to S_I in the granite (Fig. 1). This shows that while the incompetent

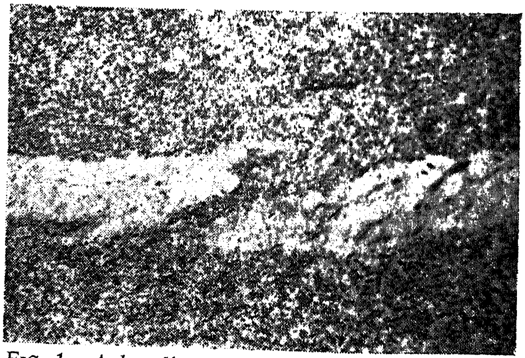

FIG. 1. A boudinaged pegmatite vein. Note flowage of pegmatitic material parallel to S_I in granite

aplites and granitic material underwent plastic deformation during F folding and amphibolite facies metamorphism, the pegmatites acted as competent bodies undergoing rupture. Thus it is evident that the above aplites and pegmatites of the granite body are pre-S_I. The post-S_I aplites and pegmatites of the granite body are free from the effects of F folding and amphibolite facies metamorphism.

The Eastern Ghat orogenic belt rocks are believed to have been involved in two phases of metamorphism during the Eastern Ghat orogenic cycle, *viz*., metamorphism (phase I) of the charnockites of Madras and Nilgiris and Khondalites closing at 2600 m.y. and a later metamorphism (phase II) of the belt accompanied by granitic and pegmatitic activity marking the culmination of the Eastern Ghat orogery at 1600 m.y.[2].

Evidences presented above suggest that the Kalpatta granite as a whole is either pre-tectonic or early syntectonic with respect to F folding and the accompanying retrograde amphibolite facies metamorphism (phase II). This metamorphism and deformation most probably coincide with the culmination of the Eastern Ghat orogeny closing at 1600 m.y. The late aplites and pegmatites must have intruded into the granite body after the culmination of the Eastern Ghat orogeny.

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1. Nair, P. K. R., *Curr. Sci.*, 1976, **45**, 379.
2. Sarkar, S. N., *Pre-cambrian Stratigraphy and Geochronology of Peninsular India*, Dhanbad Publishers, 1968.

GLYCOGENESIS IN THE LIVER OF A MURREL FISH

LITERATURE pertaining to the localization of glycogen in the body of fishes is limited and little is known about the carbohydrate metabolism in them. The present communication records for the first time the pattern of localization and significance of glycogen in the liver cells of a murrel fish, *Ophicephalus punctatus* Bloch.

Pieces of liver from the well-fed specimens, and from those undergoing fasting, were fixed in Rossman's and Helly's fixatives at 5° C for 12 to 16 hours. Irrespective of the fixative used, the sections of liver, after conventional processing, were treated with periodic acid –Schiff (PAS) reagents, and a few sections stained with Best's Carmine technique as given by McManus and Mowry[2]. The parallel sections meant for control were incubated for ¾ of an hour in the human saliva, diluted with normal saline prior to the treatment of sections with the histochemical reagents. Some of the sections were counterstained with haematoxylin to give histological differentiation of the cells.

The histochemically prepared sections showed purple-red masses in the cytoplasm of the hepatic cells, which nearly filled the whole of the cytoplasmic space (Fig. 1). The hepatic cells appeared polygonal in shape and had generally one nucleus which occupied a central position in the cell. Some of the cells possessed two nuclei of equal size. However, it was found that their appearance had no sequence in the tissues. The purple-red stain for glycogen in the cell was found to be labile on the pretreatment of sections with the saliva. The result of the histochemical preparations with PAS always corresponded very closely with those given by the Best's carmine preparations. These reactions confirmed that the stained sites in the cells were glycogen deposits.

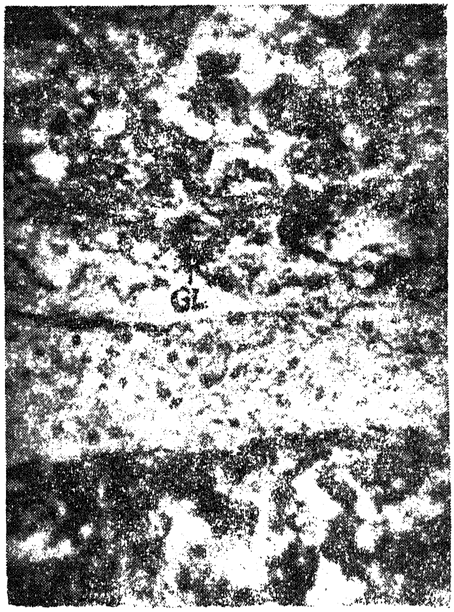

FIG. 1

The pattern of localization of the glycogen deposits in the cells was characteristic. The deposits had a granulated appearance and each granule measured 0·9 to 1·2 micron in size. However, it was interesting to find that the concentration of the deposits in the different portions of the liver, and in the different feeding conditions of the gut was not always the same. The distal and central parts of the lobes of liver had a larger concentration of glycogen in their hepatic cells than in those at the base of the liver, and the level of concentration was conspicuous in the liver of the well-fed specimens. Thus the nature of localization of the glycogenic substances in the present fish largely agreed with those of *Salmo gairdnerii*[5] and *Catla catla*[3]. However, it showed difference from the case of the mosquito fish, *Gambusia affinis*[1], for its having minute, globular and unstained lipidlike substances in between purple-red stained masses of the glycogen in the cells with the result that the aggregation of the glycogen masses was not compact in *O. punctatus*. The difference in the nature of the diet (piscivorous feeding habit of *O. punctatus*) seems to be responsible for this variation.

In an earlier investigation of this fish[4], a high level of alkaline phosphatase activity was noted in the striated border of the absorptive cells of the anterior portion (including pyloric caeca) of the intestinal tract. This provided an indirect evidence to the possibility of a vigorous phosphorylation activity in the gut-wall, and as such in the high rate of glycogenesis in the liver.

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Ranchi-8, *December* 15, 1976.

---

1. Bullock, W. L., *Acta Zool., Stockh.*, 1967; 48, 1.
2. McManus, J. F. A. and Mowry, R. W., *Staining Methods : Histological and Histochemical*, Paul B, Hoeber, Inc., 1960.
3. Saxena, S. C., *Proc. Indian Acad. Sci.*, 1966, 64, 176.
4. Shafi, Md., *Aligarh Muslim University Ph.D. Thesis*, 1973, p. 80.
5. Weinreb, E. L. and Bilstad, N. M., *Copeia*, 1955, 3, 194.

SUSCEPTIBILITY OF AN INSECTIVOROUS BAT (*RHINOLOPHUS ROUXI*) TO EXPERIMENTAL INFECTION WITH KYASANUR FOREST DISEASE VIRUS

The involvement of chiropiera in the natural cycle of Kyasanur Forest Disease (KFD) virus was first suspected when neutralizing antibodies against KFD virus was demonstrated in two species of bats, *viz.*, a frugivorous bat, *Rousettus leschenaulti* (Pavri and Singh[1], 1965) and an insectivorous bat, *Rhinolophus rouxi* (VRC, unpublished data). Subsequently, four isolations of KFD virus were made from the spleens of naturally infected *R. rouxi* and one from their argasid tick ectoparasites (Rajagoplalan *et al.*[2], 1969). Our interest was therefore drawn to investigate viraemia in this species of bat after experimental infection with KFD virus.

While embarking on an experiment of this nature it was felt necessary to maintain these bats alive in captivity. We made several attempts in this regard. At first live and killed meal worms were left in the cages for bats to feed. Since the bats did not accept this feed, a mixture containing meal worms, ripe banana, cheese and white ants mashed into a paste was force fed to bats (Sulkin, personal communication). However, the bats did not accept this feed too. A proportion of bats survived up to 4 days under laboratory conditions. Since the bats were too small to be bled daily we decided to sacrifice them at daily intervals after experimental infection.

Twenty-five bats were inoculated subcutaneously, with 3·6 dex to 8·6 dex of KFD virus (strain isolated from *Haemaphysalis spinigera*). Two to five bats were sacrificed daily subsequent to the inoculation of KFD virus. A serial tenfold dilution of blood in rabbit serum phosphate saline (RSPS) and 10% suspension of organs such as, spleen, kidney, lung, brain and salivary glands, were inoculated intracerebrally into adult swiss mice. Confirmation of KFD virus isolated from blood and organs was made by complement fixation tests.

In the first experiment eight bats received 3·6 dex of KFD virus. The level of virus in blood ranged from traces of virus on first and second post-infection (PI) days to 3·0 to 4·0 dex/0·03 ml on third and fourth days after inoculation. KFD virus was recovered from spleen, lung and brain on 2nd PI days, and spleen, lung, brain, kidney and salivary glands on third and fourth PI days. In the second experiment 17 bats received 8·6 dex of virus. The level of virus in blood ranged from 1·0 dex/0·03 ml to 3·0 dex/0·03 ml on first day to 2·7 dex/0·003 ml to more than 4·7 dex/0·03 ml on fourth PI days. Virus was isolated from spleen on first through fourth PI days.

This preliminary experiment has shown that KFD virus circulates in the blood of *R. rouxi* after experimental infection. Since the bats did not feed on the diet provided by us, they were apparently under physiological stress during the study. The dosate of virus given in one experiment was considerably high and it may be argued that viraemia recorded may not imply a replication of virus. However, in the first experiment where 3·6 dex of virus was used there was a gradual increase in the titre of virus in blood from first through fourth PI days. This would suggest that KFD virus circulates in the blood of *R. rouxi* possibly after replication.

The authors wish to express their sincere thanks to the Director, Virus Research Centre, Poona, for constant encouragement given throughout the study. Our thanks are also due to Dr. K. Banerjee for the

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---

1. Pavri, K. M. and Singh, K. R. P., *Indian J. Med. Res.*, 1965, **53**, 956.
2. Rajagopalan, P. K., Paul, S. D. and Sreenivasan, M. A.' *Ibid.*, 1969, **57**, 805.

CORRELATION BETWEEN CYTOCHROME *C* AND MYOGLOBIN CONTENTS OF THE DIFFERENT LEG MUSCLES AND M. PECTORALIS WITH REFERENCE TO SEX OF THE DOMESTIC FOWL

Factors affecting the percentage of myoglobin in muscle[1] had confirmed that a high concentration of this pigment is usually found in muscles of high physiological activity. This is of particular interest in view of the strong affinity of myoglobin for oxygen which enables it to act as an intramuscular oxygen store for the tissue's principle oxidizing catalysts, the cytochromes[2-3]. Cytochromes are heme proteins in which iron forms the core of the heme and it is the iron that is oxidized and reduced in electron transport. Keilin[4] as early as 1925 had pointed out that all aerobic cells contain cytochromes (as determined spectroscopically) and is highest in the most actively respiring cells. However, the concentrations of cytochromes has been reported both as being higher or lower in myoglobin rich or 'red' muscles than in the 'white' variety.

In the course of our investigation on the myoglobin content of certain muscles of the domestic fowl, it has been noted that certain muscles involved in walking are rich in myoglobin, fat and lipase and others contain less of these; also that these substances are less in the M. pectoralis of the fowl[6]. It was therefore thought advisable to find out if there is any correlation between myoglobin content and that of cytochrome *C* content in the leg muscles and breast muscles of the male and female domestic fowl.

The birds used were (18 months old male and female) those of Karnatak University breed. The bird was killed by decapitation and the leg as well as breast muscles were removed immediately and stored at 0° C until used. The myoglobin concentration of different muscle preparations was estimated as described elsewhere[7]. The cytochrome *C* content of the different leg as well as breast muscle was extracted and estimated according to the procedure described by Potter and Dubois[8].

The cytochromes are the natural hydrogen acceptors of the dehydrogenase system in the cell. The best known member of the cytochrome system is cytochrome *C*, the soluble cytochrome. Myoglobin is involved in the hydrogenation process and as such one can expect a correlation between the myoglobin and cytochrome *C* content in a muscle; if more of these two components occur; such a muscle is expected to be more active than others. The results given in Table I show that M. gastrocnemius pars externa and semitendinosus in the male domestic fowl show more of myoglobin and cytochrome *C* content than other leg muscles. In the female M. sartorius shows more of these substances than the two muscles M. gastocnemius and semitendinosus as well as other muscles. Though this is a sexual difference, the explanation for this has to be sought in the stress to which M. sartorius is subjected to in the female as a result of development of ovary and oviduct. In the ultimate analysis the differences between the muscles in cytochrome C and myoglobin content can be attributed to their specific individualities on the one hand, and the use or strain to which they are subjected to on the other hand; this use in males may be due to higher exercise.

Table I

Correlation between cytochrome C and Myoglobin contents of the different leg muscles and M. pectoralis with reference to sex of the domestic fowl

Muscle	microgram/gm wet wt.			
	Cytochrome C		Myoglobin	
	Male	Female	Male	Female
Sartorius	22	53*	1070	2806
Biceps femoris	14	13	1830	1742
Semitendinosus	25	22	3250	1223
Tibialis anterior	16	14	1290	1196
Gastrocnemius pars externa	70	31	4530	2657
Pectoralis	6·5	6·0	44	35

* The results are averages of three determinations in each case.

Myoglobin values are used for comparison purpose (unpublished results).

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---

1. Lawrie, R. A., *J. Agri. Sci.*, 1950, **166**, 356.
2. Hill, R., *Proc. Roy. Soc. B*, 1936, **120**, 472.
3. Millikan, G. A., *Ibid.*, 1937, **123**, 281.
4. Keilin, D., *Ibid.*, 1923, **98**, 312.

5. Fujita, A., Hata, T., Numata, I. and Ajiska, M., *Biochem. Z.*, 1939, **301**, 376.
6. Narasubhai, A. V., Kallapur, V. L. and George, C. J., *Pavo*, 1972, 10, 1.
7. —, Alexander, K. M. and George C. J., *J. Animal Morphol. Physiol.*, 1972, **19**, 83.
8. Potter, V. R. and Dubois, K. P., *J. Biol. Chem.*, 1942, **142**, 417.

REPRODUCTIVE ADAPTATIONS IN THE INDIAN RHINOLOPHID BAT, *RHINOLOPHUS ROUXI* (TEMMINCK)

THE breeding behaviour of several Indian bats has been studied[1–7] based on specimens collected from a restricted locality. However, variations in the breeding behaviour of any Indian species inhabiting different regions and occurring in markedly different climatic conditions have not been studied so far. Hence, a study of the reproduction of *Rhinolophus rouxi* at two different regions of this subcontinent was undertaken to determine if this species exhibits reproductive adaptations to different environmental conditions. The specimens were collected at frequent intervals at Bangalore (Latitude 12°, 58′, Longitude 77°, 38′) in South India and from the mountain tunnels at Khandala (Latitude 18° 52′, Longitude 71° 32′) in Western Ghats. In both the localities this species has an annual reproductive cycle, and each adult female carries a single conceptus invariably in the right uterus.

At Bangalore, the females are in oestrus during the last week of November. Unfertilized ova, stages of fertilization and early cleavage were noticed in the Fallopian tubes of specimens collected on 27th November. Early uterine morulae were found between 6th and 8th December and free unilaminar and early bilaminar blastocysts were noticed in the uteri of specimens collected between 12th and 14th December. Early implanted blastocysts were noticed in specimens collected on 21st December and the embryos with early amniotic folds were observed on 12th January. During the following weeks all pregnant females collected on any given date had embryos nearly at the same stage of development and all the females in the colony delivered their young between 26th April and 8th May.

At Khandala, the adult females collected on 13th December had each a large preovulatory follicle but hadn ot mated. Late uterine morulae were noticed in the specimens collected between 7th and 23rd January. In all the specimens collected during this period, the morula was nearly at the same stage of development. Unimplanted blastocysts were noticed in the uterus of specimens collected between 1st February and 12th February. Adult females collected on 23rd February had each an early implanted blastocyst. Progressively advanced stages of development were present in the females collected during the following weeks until 21st May when each adult female had a full term conceptus which, judging from the weight of the foetus, would have delivered within a week. The fact that all pregnant females collected on any given date after the 23rd February had embryos at nearly the same stage of development indicates that ovulation and fertilization are nearly synchronous in all the females in the colony. Since this occurs in the first week of January, it is evident that the embryos remain free in the uterine lumen between the first week of January and about the 23rd of February.

The above data lead to the following conclusions:

1. The breeding season of *Rhinolophus rouxi* commences at Bangalore about a month earlier than at Khandala.
2. The gestation period of this species is 150 ± 8 days in both places.
3. While early embryonic development of this species is considerably slowed down at Bangalore, the morula and the unattached blastocyst stages of development are unusually prolonged in the specimens at Khandala. Hence, the implantation of the embryo is delayed in the specimens at Khandala, and the embryo remains free in the uterus for about 40 to 45 days.
4. In both the localities the young are delivered just before the onset of the rainy season. Evidently, the setting of the monsoon is an important ecological factor which determines the period when the young are delivered in this species. At Bangalore, deliveries occur about 4 weeks earlier than at Khandala and this is also reflected in the time of onset of the monsoon which is about 4 weeks earlier at Bangalore than at Khandala. The delivery of the young just before the onset of monsoon is apparently an adaptation to the environment to ensure that mothers in lactation and the weaned young have a plentiful supply of insects, which are abundant during the rainy months. The gestation period is same in both the localities. It is evident that the other differences in the reproductive pattern of this species at the two localities, namely the retarded development of the early embryos at Bangalore and the occurrence of delayed implantation of the embryo at Khandala, are adaptations to the differences in the ecological factors in the two localities. The occurrence of delayed implantation in the species at Khandala can be expected since the bat comes to heat in December.

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---

1. Gopalakrishna, A., *Proc. Indian Acad. Sci.*, 1947, **26**, 219.
2. —, *Ibid.*, 1948, **27**, 137.
3. —, *Ibid.*, 1949, **30**, 17.
4. —, Thakur, R. S. and Madhavan, A., *Dr. B. S. Chauhan Commemoration Volume*, Zool. Soc. India, 1975, p. 225.
5. Madhavan, A., *Mammalia*, 1971, **35**, 283.
6. Ramakrishna, P. A., *Jour. Mysore Univ.*, 1951, **112**, 107.
7. Ramaswamy, K. R., *Proc. Nat. Inst. Sci. India*, 1961, **27**, 237.

A NOTE ON *HELICOVERPA ARMIGERA* (HUBNER) HARDWICK, A NEW GENERIC COMBINATION OF GRAM POD BORER *HELIOTHIS ARMIGERA* Hb.

For many years, the gram pod borer or tomato fruit borer, a major pest of *rabi* pulses and cotton throughout Indian continent has been referred to as *Heliothis armigera* Hubner.

Similarly in Europe and Western Countries, the corn earworms (*Heliothis zea*) and the bollworms of cotton were also considered to constitute only one cosmopolitan species, being referred to as either *Heliothis obsoleta* or *Heliothis armigera*. Recent investigations by the taxonomist of *Heliothis* fauna, however, suggested the presence of complex of the species and questioned the validity of generic name of *Heliothis* (Hardwick, 1965).

Morphologically, it is a homogeneous, group but differs sufficiently in the genetalic structures. Hardwick (1965[1] and 1970[2]) redefined the genus *Heliothis* and described a new genus *Helicoverpa*. The species of *Helicoverpa* were separated from *Heliothis dipsacea* (Linn.), type species of *Heliothis*, on the basis of the structure of both male and female genitalia and the presence of the specialised scales on the underside of the fore femur of the male, the scales being absent in the genus *Heliothis*. Structurally the genus *Helicoverpa* is readily distinguished from *Heliothis* by the possession of a multi-coiled vesica in the male and an alternately dilated and constricted appendix bursae in the female.

To determine the species complex of gram pod borer and confirm its generic nomenclature, the specimens of moths, representing a large collection made during 3 years, were sent to Dr. Hardwick, for identification. The consignment consisted of 11 specimens each differing in the colour pattern of fore and hind wings, in size and other minor details. Ten out of the eleven specimen were identified as *Helicoverpa armigera* (Hubner) and one as *Heliothis peltigera* Schiff. *Helicoverpa armigera* represented over 99% of the collection so far referred to as *Heliothis armigera* and thus observed to be the most predominent species prevalent in this part of the country.

The author is grateful to Dr. David F. Hardwick, Director, Biosystematics Research Institute, Canada, Department of Agriculture, Ottawa, Canada, for identifying the species.

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---

1. Hardwick, D. F., *Mem. Entomol. Soc. Canada*, No. 40, 1965.
2. —, *Ibid.*, No. 73, 1970.

PHOTOPERIODIC CONTROL OF FLOWERING IN GROUNDNUT (*ARACHIS HYPOGEA* L.)

Groundnut varieties have a high tolerance to photoperiodic changes since they can be grown throughout the year in this country where temperature conditions remain favourable. The crop requires higher air and soil temperatures for better vegetative and reproductive growth[1]. Precise studies regarding photoperiodic sensitivity for this crop are not made so far, although it has been reported that flowering of this crop is not influenced by photoperiod[2-4]. Some reports suggest that in groundnut flower initials already exist within the seeds even before germination and hence it does not require photoinduction[5]. It is also shown that the plant is sensitive only during the first six days after germination[6]. Hence, there is a need to examine the photosensitivity of groundnut more precisely.

The groundnut seeds of eight varieties, commonly grown in this country, *viz.*, TMV-1, TMV-2, TMV-10, J-11, Pol-1, C-148, M-145 and M-13 were sown in small plots and exposed to 6, 8, 10, 14 and 24 hrs of photoperiods from the 10th day of germination. For short day treatments (6 to 10 hrs light) plants were covered with black cloth on a special structure. For continuous light treatment (24 hrs light) 100 watt incandescent lamps were used during the night. Normal day plants (14 hrs light) were grown under natural light conditions. The appearance of first flower and 50% flowering were noted.

Changes in day length caused considerable effect on the flowering in most of the varieties. An increase in day length from 6 to 10 hrs hastened the flowering (5 to 7 days) in TMV-1, TMV-2, TMV-10, J-11 and M-13. Day length beyond 10 hrs delayed the flowering significantly. Two varieties Pol-1 and C-148 were found to be relatively less sensitive to the changes in photoperiod.

These results suggest that all the eight varieties flowered under a wide range of photoperiod (6 to 24 hrs) but flowering time was influenced by the changes in the length of photoperiod. Ten hours of light with 14 hours of associated darkness was optimum for hastening the flowering time. Shorter or longer photoperiod tended to delay the flowering. These experiments clearly show that groundnut varieties are photosensitive; hence earlier suggestions that the plant could be classed as day neutral[2-4] is not substantiated. It is interesting to note that photoperiods shorter or longer than 10 hrs delayed the flowering in most of the groundnut varieties used in this experiment.

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---

1. Bohluis, G. S. and Groot, W. De, *Neth. J. Agric. Sci.*, 1959, 7, 317.
2. Smith, B. W., *Am. J. Bot.*, 1954, **41**, 607.
3. Fortainer, E. J., *L. Mededelingen van de Landbouwhogeschool Te Wageningen*, 1957, **57**, 1.
4. Wynne, J. C., Emery, D. A. and Downs, R. J., *Crop Sci.*, 1957, **13**, 511.
5. Tetnyi, P., *Acta Agron. Acad. Hungarica*, 1957, 2, 302.
6. Rossen, A. V. and Bohluis, G. G., *Neth. J. Agric. Sci.*, 1954, 7, 302.

SPONTANEOUS CEREBRAL NOSEMIASIS IN A LABORATORY MOUSE

Encephalitozoon cuniculi, the cause of spontaneous paralysis in rabbits, has been found to affect a large number of laboratory and domesticated animals (rat, mouse, rabbit, guinea pig, hamaster, ferret, cat, dog, ox and goat), avian species (pigeon, parrot and sparrow) and man (Perrin[1], Lainson *et al.*[2], Jirovec[3], Petri[4], and Pattison *et al.*[5]). The organism was renamed as *Nosema cuniculi* by Lainson *et al.*,[2] 1964. Ray and Raghavachari[6] and Khanna and Iyer[7] described this parasite from the kidneys of rabbit and goat respectively from this country. The purpose of this note is to place on record the occurrence of *Nosema cuniculi* in the brain of a white mouse for the first time from this country.

On histopathological examination of formalin fixed tissues collected from a mouse which had died of botryomycosis affecting various interval organs, the cerebral white matter showed focal scattered areas of necrosis, infiltrated by glial cells with hyperaemic changes in the neighbouring vascular channels. Gram stained sections revealed small, rounded pseudocysts containing numerous small Gram-positive cigar-shaped parasites with rounded ends approximately $0 \cdot 1 - 1 \times 1 \cdot 5 - 2 \cdot 5\,\mu$ in size (Fig. 1). The parasites had a clearly defined nucleus with a dark staining central band and rounded pale area at the poles. They stained weakly with Giemsa but intensely by Goodpasture-Perrin technique. On the basis of morphology and staining affinity, the parasite was identified as *Nosema cuniculi* which was also confirmed by Dr. P. C. C. Garnham, F.R.S., London School of Tropical Hygiene and Medicine, U.K., during his recent visit to this Institute.

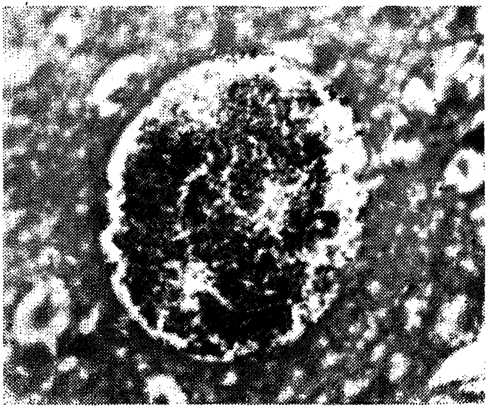

FIG. 1. Brain showing parasitic-cyst of *Nosema cuniculi*. Gram, × 1,000.

The parasites Nosema and Texoplasma are very similar and differentiation between them is based on immunological and morphological characteristics (Soulsby[8]). Morphologically Toxoplasma is larger in size, crescent-shaped and is Gram-negative. Cytoplasm is granulated with Giemsa stain and the organism stains well with haematoxylin and eosin.

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1. Perrin, T. L., *Arch. Path.*, 1943, **36**, 559.
2. Lainson, R., Garnham, P. and Killick-Kendrich, P. C. C., *Brit. Med. J.*, 1964, **22**, 470.
3. Jirovec, O., *Schweizer Arch. Tierheik*, 1967, **109**, 497; *Vet. Bull.*, 1969, **38** (Abst. 90).
4. Petri, M., *Acta Path et Microbiol. Scand.*, 1969, Supplement No. 204.
5. Pattison, M., Clegg, F. G. and Duncan, A. L., *Vet. Rec.*, 1971, **88**, 404.
6. Ray, H. N. and Raghavachari, K., *Indian J. Vet. Sci.*, 1941, **11**, 38.
7. Khanna, R. S. and Iyer, P. K. R., *Indian J. Med. Res.*, 1971, **59**, 993.
8. Soulsby, E. J. L., *Helminths, Arthropods and Protozoa of Domesticated Animals*, Pub. Baillere Tindal and Cassel Ltd., Sixth di Etion, London, pp. 746.

WHITE: A NEW COLOUR LOCUS IN *KERRIA LACCA* (KERR)

Introduction

THE white lac insect was first picked up in a F_4 progeny from a cross of two distinct races of the common Indian lac insect, *Kerria lacca* (Kerr)[1]. The discovery was considered significant because of the industrial demand for a colour-free lac resin. Later, however, it was recorded occurring in low frequencies in certain natural populations of *K. lacca*, which had suggested that this could be a recessive mutant with a selective disadvantage in its natural environment[2]. This communication reports the results of crosses of the white with the other colour forms of these insects.

Materials and Methods

The insects used in crosses with the white variant originated from the laboratory maintained homozygous stocks of (i) the wild-type crimson which produces a yellow resin, and (ii) the yellow mutant, in which the insect colour is changed to yellow, but the resin colour remains unaffected. The white produces no colour either in the insect or in its resin. The test insects were maintained on potted plants of *bhalia* (*Moghania macrophylla*) covered by a fine muslin cloth cage to control contamination and to prevent losses due to parasitic and predatory activity. The progeny was scored at the time of sexual maturity since the lac larvae, on hatching, invariably retain the colour of their mother and the change in colour produced by their own genotype becomes apparent only after they have grown for some time on the host plant, usually in the second instar[3]. Five or more progenies were scored from each class of mating.

Results

Reciprocal matings of the white and wild-type stocks of *K. lacca* produced only wild-type progeny, confirming dominance of the wild-type over white. The F_1 females were mated to the white males. Table I shows the testcross distribution in crosses of these colour forms. The parental colour forms reappeared in a proportion which did not deviate significantly from parity. The colour-free state of the white lac insect thus behaves as a simple recessive. A gene symbol *w* is proposed for the new mutant gene which blocks the production of colour in both the insect and its resin.

TABLE I

The testcross distribution of white and wild-type in crosses of the two colour forms in K. lacca

	White	Wild-type	Total
Observed	1074	1146	2220
Expected*	1110	1110	2220

* Expected if the white was a simple recessive condition.

$\chi^2 = 2\cdot34$. $P > 0\cdot1$.

Crosses of the white and yellow mutants produced wild-type progeny, confirming that the gene for white is non-allelic to that for the yellow. The white is thus the second colour locus in *K. lacca* which is shown to be subject to allelic segregation causing variation in the colour of the insect and its resin.

During the course of these experiments, the white was found to have a much reduced fecundity and survival. This explains the low frequency occurrence of white in natural populations of these insects recorded earlier[2] and reduces its utility as an insect of commerce.

Discussion.

Chemical investigation of the lac insect pigments has shown that these are anthraquinone derivatives and are of two distinct types. The one found in the insect is water-soluble and is termed as laccaic acid. The other present in the lac resin is water-insoluble, but spiriti-soluble and is referred to as erythrolaccin[4]. More recently each of these pigments is shown to be associated with a number of closely related compounds and, based on chemical evidence, a tentative biosynthetic scheme has been suggested, in which the laccaic acids and erythrolaccins are regarded as the end products and their biochemical origin is traced to a common precursor what has been termed as laccaic acid D[5,6]

The evidence presented here shows that a single gene mutation has blocked the biosynthesis of both the laccaic acids and erythrolaccins in the white variant. This confirms their common biochemical origin suggested earlier on chemical evidence. The yellow gene changes the insect colour, but leaves the resin colour unaffected, suggesting that it probably affects the independent pathway of the laccaic acids. Similarly, mutations can also affect the resin colour exclusively, and such mutations, by virtue of allowing the insect to retain its body colour, might be expected to do away with the biological weaknesses of the white mutant to help produce the much needed white lac. A knowledge of other colour mutations and their biochemical effects on the lac pigments will now be awaited with great interest for the solution of the all important colour problems in lac.

Authors are thankful to Dr. T. P. S. Teotia, Director, for encouragement.

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1. Chauhan, N. S. and Teotia, T. P. S., *Indian J. agric. Sci.*, 1973, **43**, 1086.
2. —, *Curr. Sci.*, 1976, **45**, 566.
3. —, *Indian J. Ent.*, 1967, **29**, 216.
4. Bose, P. K., Sankaranarayanan, Y. and Sen Gupta, S. C., *Chemistry of Lac*, Indian Lac Research Institute, Namkum, Ranchi, Bihar, India, 1963.
5. Bhide, N. S., Pandhare, E. D., Rama Rao, A. V., Shaikh, I. N. and Srinivasan, R., *Indian J. Chem.*, 1929, **7**, 987.
6. *Annual Report of the Indian Lac Research Institute for the year* 1967, New Delhi, Indian Council of Agricultural Research.

ALBINISM IN *XANTHOMONAS SESAMI*

Xanthomonas sesami Sabet and Dowson, the incitant of bacteriosis in sesame (*Sesamum orientale* L.), is a typical yellow-pigment phytopathogenic bacterium. The pigmentation has been observed to be a constant character of all the cultures maintained by the writer.

During a routine subculturing operation, a white but *Xanthomonas*-like colony was detected to be growing among the yellow-pigmented ones in two culture tubes. These colonies were carefully transferred to sterilized water columns and streaked on NA medium in petridishes. The pigmented colonies of the tubes were also processed similarly to serve as checks. All the colonies developed in the first case were non-pigmented but with typical colony characteristics of the xanthomonads. The check petridishes did not reveal any change of pigmentation in the colonies. The consistency of colony colour was further confirmed by growing them on various media (NA, PDA, PSA* and potato wedges) and at different temperatures (15°, 20°, 25°, 30°, 35° C). The colour pattern of the colonies of non-pigmented and pigmented forms did not show any deviation.

On young and healthy host plants, both the forms produced typical symptoms of the disease on inoculation. The white and the yellow forms were compared for their morphological, cultural and biochemical characteristics and bacteriophage susceptibility. A close similarity of characters was evident between the chromogenic and achromogenic forms of the cultures. It was, thus, suggestive that the non-pigmented forms had arisen from the yellow-pigmented cultures by mutation. The achromogenic (albino) forms were, therefore, identified as 'albino' strains of *X. sesami*.

In 1969, the writer[3] recorded the development of an 'albino' form in *X. uppalii* Patel. The present investigation provides two more evidences for the development of 'albino' mutants in xanthomonads.

In an earlier article[3], the writer had suggested that only those phytopathogenic non-pigmented bacterial forms should be accepted as 'albino' xanthomonads, whose original pigmented *Xanthomonas* species are traceable in nature and such forms should be specified as achromogenic (albino) strains of the existing chromogenic *Xanthomonas* spp. It would not be, therefore, inappropriate to mention that the recent efforts to group the colourless, *Xanthomonas*-like bacteria, which occur only in achromogenic forms in nature, such as *X. padalii*[5], under the genus *Xanthomonas*, need further careful taxonomic assessment so that re-occurrence of the dilemma created by the grouping of 7 non-pigmented phytopathogenic bacterial forms as species of the genus *Xanthomonas*[1], could be avoided.

The genus *Xanthomonas* was created by Dowson[2] in 1939 in order to separate pigmented and non-pigmented polar flagellated phytopathogenic bacteria, grouped together as species of *Pseudomonas* by Lehman and Neumann[5]. The main distinguishing criterion of the genus *Xanthomonas* was the production of a typical yellow coloured growth by the bacterial mass on nutrient glucose agar and sterilised potato slants. The criterion still acts as the most conspicuous phenotypic feature of the genus *Xanthomonas*. The attempt to identify such non-pigmented bacterial forms as species of *Xanthomonas*, for which the evidences of origin from pigmented xanthomonads are not yet available; therefore, cannot be justified as natural and practicable. It would be less confusing if these organisms are accommodated as species of *Pseudomonas*, a genus which is undoubtedly more heterogeneous than *Xanthomonas* and which seems to be composed of forms that have no apparent phylogenetic relationship. The approach would prove more rational, logical and systematic and also helps to maintain the remarkable uniformity of the genus *Xanthomonas* undisturbed.

The author is grateful to Dr. V. V. Chenulu, Head of the Division of Mycology and Plant Pathology, for providing facilities.

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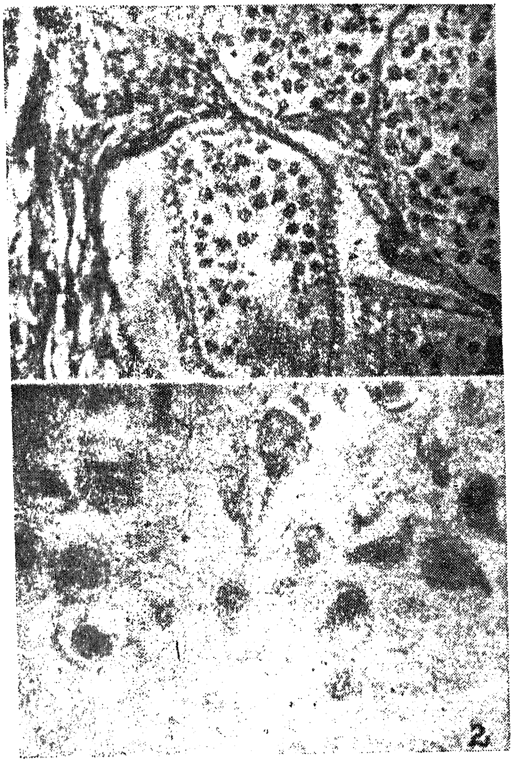
* Composition of peptone sucrose agar (PSA) medium; peptone 5 g, sucrose 20 g, Na_2HPO_4 2 g, $FeSO_4$ 0·5 g, Ca $(NO_3)_2$ 0·5 g; and 1000 ml water.

1. Breed, R. S., Murray, E. G. D. and Smith, N. R., *Bergey's Manual of Determinative Bacteriology*, 7th ed., Williams and Wilkins Co., Baltimore, 1957, p. 152.
2. Dowson, W. J., *Zbl., Bakt. II*, 1939, **100**, 177.
3. Durgapal, J. C., *Curr. Sci.*, 1969, **38**, 391.
4. Lehmann, K. B. and Neumann, R. O., *Bakteriologische Diagnostik*, J. F. Neumann, Munich 1896-1927.
5. Patel, P. N. and Jindal, J. K., *Indian Phytopath.*, 1972, **25**, 318.

IN SITU GERMINATION OF POLLEN GRAINS IN *PINUS ROXBURGHII* SAR.

KONAR[1] studied the morphology and embryology of *Pinus roxburghii* and gave its comparative account with *Pinus wallichiana*. According to him the pollen grains are shed at the four-celled stage and they germinate forthwith after pollination during March and April. However, the Fig. 14 given by him representing the oldest pollen grain displays the three-celled condition and it is the wall of the pollen grain that was labelled as the tube nucleus. It does not show the tube nucleus, although the disintegrated first prothallial cell, healthy prothallial cell and the antheridial cell are shown.

In our material collected during January 1971 from the Government Botanic Garden, Ooty, we observed the germination of pollen grains *in situ* (Figs. 1, 2). The object of this note is to bring to light that such an interesting feature could be observed in 25–30% of the pollen grains in the microsporangia. At this stage some of the pollen grains showed two prothallial cells already disintegrated and absorbed and the generative and the tube cells in healthy condition, while in others the antheridial cell alone could be made out (Fig. 2).



FIGS. 1–2. *Pinus roxburghii*. Fig. 1. L.s. part of the male cone showing the microsporangia and the pollen grains, × 70. Fig. 2. germinating pollen grains in the microsporangium, × 300.

The authors are thankful to Dr. (Mrs.) N. Lakshmi for taking the microphotographs.

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1. Konar, R. N., *Phytomorphology*, 1960; 10, 305.

SOME NATURAL ENEMIES OF *SAISSETIA COFFEAE* ON COFFEE IN INDIA

AMONG Coccids attacking coffee in India, the brown bug *Saissetia coffeae* (Walker) (Homoptera, Coccidae), is reckoned as a minor pest. But occasionally severe attacks occur, resulting in considerable loss of crop.

Very little information is available on natural enemies of this pest in India. Coleman and Kannan (1918) mention about a number of hymenopterous parasites and a predatory maggot as natural enemies of *S. coffeae*, but do not give the identity of these. The only identified insect which they have recorded is *Eublemma* sp. (Lep., Noctuidae) which feeds on eggs of *S. coffeae*.

During surveys made in 1975 and 1976, the author recorded for the first time the folllowing natural enemies on *S. coffeae* attacking coffee in India:

PARASITES

Hymenoptera: Aphelinidae.

1. *Coccophagus cowperi* Girault, recorded at Central Coffee Research Insitute in February–March 1975 and on Pulney Hills, Tamil Naidu, in June 1975.
2. *C. anthracinus* Compere, recorded at Central Coffee Research Institute in February–March 1975.

Encyrtidae

3. *Encyrtus lecaniorum* (Mayr) recorded at Central Coffee Research Institute in February 1975 and on Pulney Hills in June 1975.

Parasites 1 to 3 were internal and solitary.

PREDATORS

Diptera: Cecidomyiidae

4. *Dicrodiplosis fulva* (Felt), recorded from Kachige Estate, Chikmagalur District, Karnataka, in April 1976. K. M. Harris (personal communication) writes: "Until now this species has been known from only three adult specimens. It was described from Peradeniya, Sri Lanka where it was found on *Saissetia nigra* in 1974. The only subsequent record is from *Saissetia* sp. on clove in Tanzania in 1950. The larvae are probably predators on eggs and on adult and immature *Saissetia*."
5. *Megommata* sp., recorded on Pulney Hills in June 1975, feeding on eggs.

Neuroptera: Chrysopidae

6. *Chrysopa* sp. This unknown species was recorded on Pulney Hills in June 1975 feeding on nymphs.

Coleoptera: Coccinellidae

7. *Cryptolaemus montrouzieri* Mulsant feeding on nymphs and eggs on Pulney Hills in August 1975. The predator was extremely scarce, but it may be mentioned that *C. montrouzieri* was first introduced into Pulney Hills in India from Australia in 1898 (Puttarudriah *et al.*, 1952; Mayne, 1953). Although the predator was subsequently recovered from several other localities, until now, there was no other record of it from Pulney Hills.

The author expresses his thanks to the Director, Commonwealth Institute of Entomology, London, for determining the parasite and predator species 1 to 6.

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---

1. Coleman, Leslie, C. and K. Kunhi Kannan. "Some scale insect pests of coffee in South India" Department of Agriculture, Mysore State, *Entomological Series—Bulletin No.* 4, 1918, p. 55–61.
2. Mayne, W. W. *Cryptolaemus montrouzieri* Mulsant in South India. *Nature*, **172**, 1953, 85.
3. Puttarudriah, M., ChannaBasavanna, W. P. and Krishna Murti, B. G. "Discovery of *Cryptolaemus montrouzieri* Mulsant (Coccinellidae, Coleoptera Insecta) in Bangalore, South India," *Nature*, 1952, **169**, 377.

VEGETATIVE PROPAGATION OF SANDAL, *SANTALUM ALBUM* L.

VEGETATIVE propagation is an important method of propagating tree species, particularly when the original characteristics of the parent tree are required to be retained. There are several methods of vegetative propagation[1]—through leaves, through stem cuttings, through root cuttings, through air-layerings, etc., and some of these methods have been tried with sandal during the course of the past few years with varying degrees of success and the results are now presented in the present note.

Air-layering experiments were carried out in different seasons, but only those which were conducted during March–April proved successful. For these experiments branches with approximately 2 cm in diameter were chosen. From them the bark was carefully removed all around so that the ring was about 1 cm in width. The rings were dusted with 0·5 g of serodix B and covered with fine sphagnum moss. Watering of the "wounds" was regularly done at periodic intervals, except during March–April when there were frequent showers in Bangalore. After 35–45 days callus formation was observed in 95% of the treated wounds and in 15–20% of the cases small roots emerged out. When the roots developed further to about 8 cm, the branchlets were separated from the main tree, cutting them at about 15 cm towards the bole from the air-layer. Each rooted branch, thus separated, was provided with a host plant and planted in a prepared fit or pot. About 50% of the rooted branch cuttings survived.

Sandal produces a large number of root suckers, which suggests that it can be propagated vegetatively through root cuttings. For initiation of suckers, a trench of about 30 cm wide and 30 cm deep was dug around a mature tree at about 1·5 metres from the trunk. In about 30–40 days the radiating roots, cut across during trenching, put forth shoot primordia from the cut ends, more profusely on the cut portion away from the tree. At this stage incisions were made on the root at about 5 cm from the shoot primordias, peeling off a small portion of the bark all around. To the debarked portion, a solution of indole acetic acid (IAA, 100 ppm) was applied and covered with fine compost, after planting a host plant very near the incision. In about 6 to 8 months, the root system developed very vigorously with roots protruding from the incised portion and haustorising on the host plant. At this stage the new plantlet was severed from the rest of the original root, dug out carefully along with the host plant and planted in a pot. The survival rate was 50 to 60%. It may be incidentally mentioned that indole butyric acid was not very effective in inducing the root formation in the above experiments.

Alternatively, in the above experiments, soon after the appearance of the shoot primordia the shooted root was cut off, away from the shoot initials, at a distance of 5 to 10 cm. The cuttings thus obtained were treated with root-promoting hormones, by immersion in a 50 ppm solution for a period of 2 hours. After the treatment the cuttings were planted in pots along with suitable host plants. During the course of 5 to 6 months, both the shoot and the root systems developed very well, the roots having already established haustorial connections with the roots of the host plant. Of all the hormones tried in these experiments, IAA and NAA were found to be the best for the root initiation and development. Further, it was found that a mixture of these two hormones taken in equal quantity was found to be more effective (50 to 60% success) than either of the hormones used alone (40% success). Propagation through stem cuttings did not succeed at all, whichever hormone was used and in whatever concentration.

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1. Mahalstade, J. P. and Haler, E. S., *Plant Propagation*, John Wiley and Sons, New York, 1959.

INHERITANCE OF PIGMENTATION OF COWPEA

Inheritance studies in cowpea were first reported by Spillman[1] in 1911 who investigated the hilum colour. Saunders[2-4] investigated on genetics of seed coat, flower, plant and pod colours, mutations and linkages and suggested probable linkage of black seed to one or more genes for early maturity. The identification of such linkages between qualitative and quantitative characters in crop plants definitely assumes new lines of research both from practical and theoretical points of view.

Although some preliminary information is available on different patterns of pigmentation on vegetative parts as reported by various workers[5-7], the genetics of this character are not well understood. From the available literature, it appears that there is no information on the inheritance of scattered purple pigmentation on various plant parts in comparison to non-purple pigmentation.

Two varieties of cowpea, *viz.*, Russian Giant (non-pigmented and Chinese Red (pigmented) were crossed during 1975 *rabi* and F_1 along with parents were studied during 1976 summer season. Seeds of parents, *i.e.*, Chinese Red, Russian Giant and their F_1 and F_2 generation material were grown in the botanical garden of Agricultural College, Dharwar, Karnataka Satte, during *kharif* season of 1976. Observations were recorded on individual F_2 plants for pigmentation. Chi-square analysis was done to study the mode of inheritance. Russian Giant is completely devoid of pigmentation on any part of the plant as a result the plant was light green in colour, while Chinese Red is characterised by the presence of purple pigmentation on different parts, *viz.*, nodal region of the main stem where the secondary branches arise, base of the tertiary branches, base of the peduncle and at the base of the stalk of trifoliate leaves. The F_1 was resembling Chinese Red indicating purple pigmentation as dominant over green.

The mode of segregation for purple and green pigmentations can be seen clearly in Table I, where F_2 segregated into two phenotypic classes— (i) purple and (2) light green. The computed value for chi-square was not significant for 15:1 ratio which indicated that two independently segregationg non-allelic duplicate dominant genes are operating in the inheritance of this trait. Thus genes P_1 and P_2 either singly or in combination influence purple pigmentation without cumulative effect and absence of these two genes results in non-purple pigmentation, *i.e.*, green. This appears to be the first report on the duplicate nature of inheritance of this trait.

Table I

Inheritance of Pigmentation in Cowpea

Sl. No.	Phenotypic classes	Observed	Expected	O – E	$(O - E)^2/E$	D.f.	$\Sigma \frac{(O-E)^2}{E}$	P
1.	Purple	1343·00	1350·90	–7·90	0·0462	1	0·7461	0·30–0·50
2.	Light green	98·00	90·6	+7·94	0·6999			

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1. Spillman, W. J., *Am. Na.*, 1911, **45**, 513.
2. Saunders, A. R., *S. Afric. J. Agri. Sci.*, 1959, **2** 285.
3. —, *Ibid.*, 1960 *a*, 3, 141.
4. —, *Ibid.*, 1960 *b*, 3, 327.
5. Anonymous, *Pulse Crops of India*, 1st Ed., ICAR Publications, New Delhi, 1970, p. 201.
6. Krishnaswamy, N., Nambiar, K. K. and Mariakulandai, A., *Madras agric. J.*, 1947, **32** (7 and 8), 145.
7. Rachie, K. O. and Rawal, K. N., *Tech. Bull., IITA*, Nigeria, 1976, **5**, 4.

NOTE ON INHERITANCE OF SEED COLOUR AND PURPLE EYE ON STANDARD PETAL IN HORSEGRAM (*DOLICHOS BIFLORUS* L.)

GENETIC studies in horsegram have been very few. Rangaswami Ayyengar *et al.*[1] and Sen and Bhowal[2] found that purple pigment in different parts of the plant and the black colour of the seed are controlled by one gene which is dominant over its allele responsible for the absence of such pigmentation in different parts and brown seeds. The present study deals with the inheritance of purple eye in the middle of standard petal and seed colour. The mode of inheritance of these two characters is reported here for the first time.

TABLE I

Segregation of Characters in F_2

Cross	Characters	Observed segregation				Ratio	X^2	P
		Dominant	Recessive					
EC 7460 × Makari shore	Seed colour	99	31			3:1	0·09	0·70–0·80
	Purple eye on standard petal	96	34			3:1	0·09	0·70–0·80
	Joint segregation of the two characters	AB*	Ab	aB	ab			
		72	27	24	7	9:3:3:1	0·46	0·90–0·95
		Dominant	Recessive					
EC 7460 × CO·1	Seed colour	157	55			3:1	0·11	0·70–0·80
	Purple eye on standard petal	155	57			3:1	0·40	0·50–0·70
	Joint segregation of the two characters	AB	Ab	aB	ab			
		115	42	40	15	9:3:3:1	0·52	0·90–0·95
		Dominant	Recessive					
Pooled F_2	Seed colour	256	86			3:1	0·004	0·95
	Purple eye on standard petal	251	91			3:1	0·47	0·30–0·50
	Joint segregation of seed colour with purple eye	AB	Ab	aB	ab			
		187	69	64	22	9:3:3:1	0·54	0·90–0·95

*AB = Black mottled seed, purple eye present; Ab = Black mottled seed, purple eye absent; aB = Brown seed, purple eye present; ab, Brown seed purple eye absent.

The culture EC. 7460, which has black-mottled seeds and no purple eye on the standard petal, was crossed with two brown seeded cultures *viz.*, 'Machari-shore' and Co. 1; both having a prominent purple eye on the standard petal. The F_1 plants raised in 1974–75, showed black-mottled seed and prominent purple eye, suggesting that black-mottled colour of the seed and presence of purple eye are dominant over brown seed and absence of purple eye on stnadard petal. The F_2 generation was grown in 1975–76 and the segregation data clearly indicated that the black-mottled colour is governed by a single dominant gene B^m, and that the presence or absence of purple eye is also monogenically controlled and the gene symbol is designated as *Pe* (Table I). The joint segregation of these two characters showed that they are independently inherited (Table I).

It is suggested that the purple eye on the standard petal can be used as a marker gene.

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---

1. Rangaswami Ayyengar, G. N., Krishna Rao, P. and Seshadri Sarma, P., *Madras Agric. J.*, 193 12, 200.
2. Sen, N. K. and Bhowal, J. G., *Indian J. Genet.*, 1959, **19**, 228.

PROTECTIVE ACTION OF SOME FUNGICIDES AGAINST APPLE ROT CAUSED BY *PENICILLIUM EXPANSUM* (LINK) THROM.

APPLE ROT is a storage disease. This disease deteriorates the keeping quality of apples within a very short period after the harvest. Usually apples could not be stored more than a month under ordinary storage conditions due to apple rot disease. It is believed that this disease may be caused due to a number of pathogens, of which *Penicillium expansum* (Link) Throm. seems to be most important[1,2,4,5]. In a preliminary survey made at this laboratory, it has been possible to identify the presence of *P. expansum* in most of the rotted apples collected from the local markets. Considering the significance of *P. expansum* in reducing the storage life of apple, work has been undertaken to find out the protective effect of some fungicides against this pathogen.

P. expansum was isolated from the rotted apples collected from the local market. The identification of the pathogen was confirmed by comparing the vegetative and reproductive characters of the isolates with the characters described by Gilman[3]. The isolates after identification, were maintained on potato-dextrose-agar slants at 26° C.

Fresh scab free apples (Red Delicious) were collected from the market and divided into two batches. One batch of fruits was pretreated with the fungicides before inoculation with the pathogen and the fruits of the other batch were first inoculated with the pathogen and then treated with the fungicides. Three fungicides namely Bavistin [2-(Methoxycarbomoyl)-benzimidazole], Allisan [2-6 dichlor-4-nitro aniline] and Difolatan cis-N-(1, 1, 2, 2-tetrachloroethylthio)-4-cyclohexane-1, 2-dicarbaximida] at three different concentrations namely 0·1%, 0·2% and 0·3% were tested. The fruits were dipped in the respective fungicidal solutions for 3 minutes. To inoculate the fruits with the pathogen, a wound of approximately 12 mm in diameter was made with a sterile knife. Inoculum was obtained from a fresh culture of the isolate and aseptically introduced into the wound. The wounded portion was then covered back with the partially removed skin of the fruit. The inoculated fruits were then incubated at 26° C and the rotting percentages of the samples were determined upto 15 days.

When the samples were observed after 7 days of incubation, the average rotting per cent of the control fruits was found to be 12·1. In the case of both the pre-inoculation and post-inoculation, the average rotting percentages with different treatments were found to be significantly lower than those with the control. When Bavistin was applied at 0·1%, 0·2% and 0·3% as pre-inoculation treatment the respective average rotting per cents were 2·1, 5·0 and 2·8 but the variations found due to different concentrations were not statistically significant. The rotting percentages with different concentrations of Allisan and Difolatan were slightly higher than that found with the different concentrations of Bavistin. The rotting per cent with 0·1, 0·2 and 0·3% Allisan were 7·1, 6·5 and 7·0 respectively, the differences observed, however, were statistically insignificant. The rotting per cents with 0·1, 0·2 and 0·3% Difolatan, were 6·0, 6·5 and 6·5; the differences observed in these cases also were not statistically significant.

In the case of the post-inoculation treatments, the rotting per cents with 0·1, 0·2 and 0·3% Bavistin were 7·0, 5·0 and 6·6. The variations observed with respect to the different concentrations of the fungicides, were not satistically significant. The rotting per cents with 0·1, 0·2 and 0·3% Allisan were 8·1, 7·8 and 7·8 and that with 0·1, 0·2 and 0·3% Difolatan were 6·3, 7·1 and 7·6. The variaions were also statistically insignificant.

When the samples were observed after 15 days of incubation, 100% rotting was found in the control. The rotting percentages in the treated samples' however, were significantly lower in the treated case and did not exceed 28·3%. In the case of the pre-inoculation treatments with 0·1, 0·2 and 0·3% Bavistin, the average rotting per cents were 6·6, 7·0 and 3·0; the variations observed were not, however, statistically significant. In the case of the treatments with 0·1, 0·2 and 0·3% Allisan, the respective average percentages of rotting were 25, 20 and 28·3 and that with 0·1, 0·2 and 0·3% Difolatan were 20, 12 and 16·6 respectively. The variations observed with the different concentrations of both the fungicides were statistically insignificant.

In the case of the post-inoculation treatments, the average rotting per cents with 0·1, 0·2 and 0·3% Bavistin, were 22·6, 11·6 and 11·6; the rotting per cent with 0·1% Bavistin was significantly higher than that with 0·2 and 0·3% Bavistin. The average rotting per cents with 0·1, 0·2 and 0·3% Allisan were 28·3, 26·6 and 28·3 respectively, the variations observed in these treatments, however, were not statistically significant. The average rotting per cents with 0·1, 0·2 and 0·3% Difolatan were 26·6, 26·6 and 16·6 respectively. The average rotting per cen with 0·3% Difolatan, was significantly lower than that observed with 0·1 and 0·2% of that fungicides.

When the rotting per cents of the fruits of pre-inoculation treatments were compared with those of the fruit of post-inoculation treatments, less rotting was found with Bavistin. From this experiment, it is apparent that dipping fruits in Bavistin at even 0·1% concentration, may protect the fruits from the rot caused by *P. expansum* to a large extent.

Grateful thanks are due to Dr. C. Sen, Reader, Department of Plant Pathology, Bidhan Chandra Krishi Viswa Vidyalaya, for kindly supplying the fungicides used in this experiment.

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1. Borecka, H., *Acta Agrobot.*, 1962, **12**, 67.
2. Borecki, Z. and Profic, H., *Ibid.*, 1962, **12**, 79.
3. Gilman, J. C., In *soil Fungi*, Iowa State University Press, 1957, p. 275.
4. Heald, F. D. and Ruehle, G. D., *Wash. Agril. Expt. Sta. Bull.*, 1931, **253**, 48.
5. Nezzetti, A., *Rapp. gen. Congr., Poml.*, Florence Ferrare, 1958, p. 54.

ON THE VARIATIONS IN THE DEGRADATION OF CARBOFURAN BY THREE SOIL FUNGI

GRANULAR insecticides are applied to soil in larger quantities to control insect pests. Unless degraded subsequently these insecticides may pose health hazards. Microorganisms have been known to play a major role in the degradation of pesticides. Although considerable amount of work has been done on the effect of insecticides on microbial population[1-4], not much information is available on the extent of degradation of insecticides, particularly carbamates by different soil microorganisms. The present note communicates the degradation of carbofuran (N-Methyl-2, 3-dihydro-2, 2-dimethyl-7-benzo furanyl carbamate) and formation of its intermediary compounds by three different soil fungi.

Fungal cultures, *viz.*, *Aspergillus niger*, *Trichoderma viride* and *Helminthosporium* sp. isolated from garden land soil of Tamil Nadu Agricultural University Experimental Farm, by enrichment culture method were multiplied on potato dextrose agar. Discs of uniform size of fungal cultures were added to Czapek's broth containing 20 ppm of carbofuran. Residues of carbofuran and hydroxy carbofuran, an intermediary compound, present in the culture filtrate were estimated at weekly intervals after harvesting the fungal mat following the TLC procedure described by Metcalf *et al.*[5].

The rate of degradation of carbofuran by different fungi increased with incubation time (Table I). Among the fungi tested, *Helminthosporium* sp. showed greater ability to degrade carbofuran than *T. viride* and *A. niger*. *Helminthosporium* degraded 70 and 77·5 per cent of carbofuran during the first and second week, respectively, and there was no carbofuran residue at all during the third week indicating the complete degradation. Such a complete degradation of insecticides by microorganisms have been reported earlier with other groups of insecticides, *viz.*, chlorinated hydrocarbons and organophosphates[6-8], although informations are limited with carbamates.

TABLE I

Degradation of carbofuran by some soil fungi

(Initial level of insecticide 20 ppm)

Organism	Quantity of carbofuran detected (ppm)			Quantity of hydroxy carbofuran detected (ppm)		
	7th day I	14th day II	21st day III	I	II	III
Aspergillus niger	15·0	6·0	4·5	0·6 (12·0)	0·75 (5·3)	0·3 (2·0)
Helminthosporium sp.	6·0	4·5	0·0	0·3 (2·1)	0·3 (2·0)	..
Trichoderma viride	9·0	6·0	6·0	0·3 (2·7)	0·3 (2·1)	0·3 (2·1)

Figures in the paranthesis denote the percentage formation of hydroxy carbofuran from degraded carbofuran.

The results also revealed that all the organisms were capable of forming an intermediary compound, hydroxy carbofuran. The formation of intermediary compounds bears certain amount of significance, since, sometimes, it is more toxic than the parent insecticide[10]. An analysis on the percentage of formation of hydroxy carbofuran from the degraded carbofuran by different organisms revealed that *Helminthosporium* sp., an efficient carbofuran degrading organism converted only 2·1 per cent as compared to 12·0 per cent by *Aspergillus niger* in the initial stage. The rest of the degraded products could not be identified as they formed part of unknown spots. Further, the compounds conjugated with glucose or other large molecules would not have been extracted with solvents and presumably, all these would have contributed to the unaccounted metabolites other than hydroxy carbofuran[5]. The present study thus reveals that soil microflora vary in their ability to degrade insecticides and further suggest that greater the rate of degradation lesser the amount of intermediary compounds formed.

The authors record their sincere thanks to Dr. G. Rangaswami, Vice-Chancellor, Tamil Nadu Agricultural University, Coimbatore, for critically going through the manuscript and helpful suggestions.

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---

1. Lichtenstein, E. P. and Schulz, K. R., *J. Econ. Entomol.*, 1964, **57**, 618.

2. Ko, W. H. and Lockwood, J. L., *Can. J. Microbiol.*, 1968, **14**, 1069.
3. Robson, H. and Gunner, H. B., *Pl. Soil*, 1970, **33**, 613.
4. Sreenivasalu, S. and Rangaswami, G., *Indian J. Microbiol.*, 1973, **13**, 89.
5. Metcalf, R. C., Fukuto, T. R., Collins, Berch, K., Aziz, El, Munoz, R. and Cassil, C. C., *J. Agr. Food. Chem.*, 1968, **16**, 300.
6. Raghu, K. and Mac Rae, I. C., *Science*, 1966, **154**, 263.
7. Anderson, J. P. E., Lichtenstein, E. P. and Whitingham, W. F., *J. Econ. Entomol.*, 1970, **63**, 1595.
8. Sethunathan, N., *Res. Rev.*, 1973, **47**, 143.
9. Matsumura, F. and Boush, G. M., *J. Econ. Entomol.*, 1968, **61**, 610.
10. Menzie, C. M., *Metabolism of pesticides an update*, 1974.

INCREASE IN AMMONIA IN THE ROOT EXUDATE OF ZINC DEFICIENT TOMATO SEEDLINGS

ZINC has been established to be one of the essential nutrients for plants[1,2]. It is involved in the action of pyridine nucleotide dependent dehydrogenase[3], metabolism of tryptophan[4] and IAA[5], peptidases[6,7,8] and protein biosynthesis[9,10]. Under the zinc deficient condition, free amino acids and amides—in particular the glutamine, asparagine, glutamic acid and aspartic acid—have been found to accumulate in tissue[11]. As these metabolites are the first products of ammonia fixation, their accumulation may lead to a check on the further assimilation of ammonia. This may, therefore, result in the possibility of ammonia accumulating in the tissue, which may at higher concentration become toxic to the tissue itself. Alternatively the accumulated ammonia could be released through roots of the plant in the medium. In the nitrogen fixing organism[12] ammonia has been reported to be released in the medium. Thus the present preliminary study was carried out to see the fate of ammonia formed in a zinc. deficient tomato seedling.

Fifteen days old seedlings of tomato having similar physical appearances were taken for this study. The control seedlings were transferred to a modified nutrient medium of Hewitt[13] in which ammonium molybdate was replaced by sodium molybdate. Another batch of seedlings was transferred to modified nutrient medium, in which zinc sulfate was committed to induce zinc deficiency in the plants. The medium was devoid of ammonium salts for facilitating the estimation of ammonia in the exudate. The nutrient medium was further treated by the procedure described by Nichols[14] to minimise the zinc present as contaminants. The nutrient medium was replaced and aerated each day. In each replicate three seedlings in 50 ml of the medium were grown for ten days. The volume was maintained by the addition of fresh medium throughout the period of experiment. When the plants growing in zincless medium started showing signs of zinc deficiency, ammonia was measured in the medium by Nessler's reagent[15] at intervals of eight hours.

The results as summarised in Fig. 1 show that the ammonia concentration increases in nutrient medium deficient in zinc at a faster rate as compared to the normal medium (it is nil or in traces). As the nutrient medium did not contain ammonium salts, the data indicate that the zinc-deficient plants have released ammonia into the medium through the roots. Furthermore, as the nitrogen source in the medium was nitrate and in zinc deficiency products of ammonia assimilation, *i.e.*, glutamic acid, glutamine, etc., are accumulated[11] it appears that the nitrate reduction to ammonia has taken place, but further conversions have been blocked.

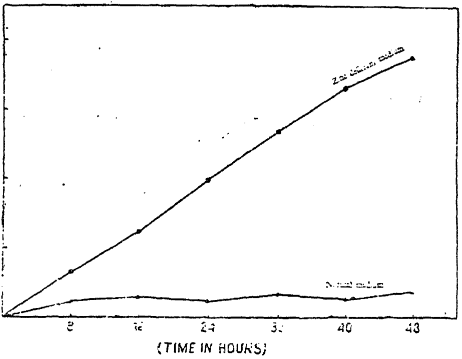

FIG. 1

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1. Maze, P., *Compt. Rand, Acad. Sci.*, 1915, **160**, 211.

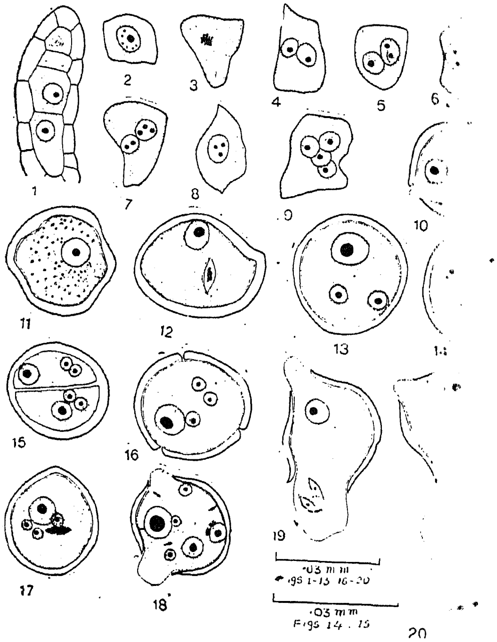
2. Sommer, A. L. and Lipman, C. B., *Plant Physiol.*, 1926, 1, 231.
3. Hoch, F. L. and Vallee, B. L., In: *Trace Elements*, eds., C. A. Lamb, O. G. Bentley, and J. M. Bettie, Academic Press, New York, 1958, p. 337.
4. Nason, A., Kaplan, N. O. and Colowick, S. P., *J. Biol. Chem.*, 1951, 188, 397.
5. Tsui, C., *Am. J. Bot.*, 1948; 35, 172.
6. Vallee, B. L. and Neurath, H., *J. Biol Chem.*, 1955, 217, 253.
7. Yudkin, W. H. and Fruton, J. S., *Ibid.*, 1947, 170, 421.
8. Linderstrom Lang, *Z. Physiol. Chem. Hoppe Seyler's*, 1934, 224, 121.
9. Wacker, W. E. C. and Vallee, B. L., *J. Biol. Chem.*, 1959, 234, 3257.
10. —, *Biochemistry*, 1962, 1, 359.
11. Possingham, J. V., *Australian J. Biol. Sci.*, 1956, 9, 539.
12. Zeltich, I., Rosenblum, E. D., Burris, R. H. and Wilson, P. W., *J. Biol. Chem.*, 1959, 191, 295.
13. Hewitt, E. J., *Commonwealth, Bur. Nort. Plantation Crops* (Great Britain), Tech. Commn. No. 22, 1952.
14. Nichols, D. J. D., In: *Physiology*, ed., F. C. Stewart, Academic Press, New York, London, 1963, p. 370.
15. *Official Methods of Analysis of the A.O.A.C.*, 1955, p. 575.

A NOTE ON THE EMBRYOLOGY OF *MARIANTHUS BIGNONACEUS* F. MUELL.

THE members of Pittosporaceae have not received much attention from the point of embryology. Davis[1] (1966) reviewed the earlier work in the family. Subsequently, Sheela and Narayana[9] (1966) and Narayana and Sundari[7] (1976) studied a few taxa. The present paper deals with the embryology of *Marianthus bignonaceus*, F. Muell., highlighting certain abnormalities hitherto unrecorded in the family.

The male archesporium is plate-like and 3–4 cells wide. The anther shows an epidermis, hypodermis which develops characteristic thickenings, two middle layers, which become crushed during development and a tapetum which is of the secretory type. The cells are uninucleate to start with (Fig. 2). By the time the pollen mother cells begin to undergo meiosis, the tapetal cells enlarge and their nuclei begin to divide resulting in a multinucleate condition. The cytoplasm becomes vacuolated. The nuclear divisions are followed by their fusion resulting in syntapetal nuclei. These syntapetal polyploid nuclei show varying number of nucleoli (Figs. 3–9).

As a result of meiosis four microspores are formed in the pollen mother cell. Cytokinesis takes place by furrowing and pollen tetrads show tetrahedral arrangement. The pollen grains are 3-colporate and are 3-celled at the sheddin (Figs. 10–13).



FIGS. 1–20. *Marianthus bignonaceus.* 1. Linear megaspore tetrad. Figs. 2–9. Tapet showing nuclear divisions and fusions. Fig Uninucleate pollen grains; note black bodies in Fig. 11. Fig. 12. Two-celled polle Fig. 13. Three-celled pollen grain. Figs. Double pollen grains. Figs. 16–18. Polle showing 4 and 6 nucleate condition. Fig Germinating pollen grains.

In several anthers abnormal pollen grai noticed. The microspore nucleus divides in the formation of two equal cells which within the common spore wall. Each cell double pollen grain behaves like a norm grain and divides forming a lenticular p cell on the common wall separating the t (Fig. 14). The generative cell in du becomes liberated into the vegetative c (Fig. 15). Such double pollen grains were earlier in *Cuscuta* (Fedortschuk[2], 1931), S (Mohrbutter[5], 1936), Podostemaceae (M 1913; Mukkada[6], 1962) and Cyperace 1962).

Besides the double pollen prains, mi with 2–6 nuclei have also been observed. the number of the nuclei in each pollen two; they are of the same size, and ca distinguished into the vegetative and generati Probably they may have been formed as a the division of the microspore nucleus. W number of nuclei is 3, 4, and 6, they difference in size (Figs. 16–18). In such instances it was difficult to decide whether the nuclei were formed by the division of the vegetative or the generative nucles. Such multinucleate pollen grains have been reported in *Jasminum, calophyllum* and *Jasminum angustifolium* (Maheswari Devi[4], 1975).

Besides the nuclei, some of the multinucleate pollen grains show deep staining chromatin-like bodies within the cytoplasm (Figs. 17 and 18). In some pollen grains a deposition of black granular bodies of unknown nature has been noticed (Fig. 11).

In some of the anthers *in situ*, germination of pollen grains was observed (Figs. 19, 20). All the germinating pollen grains were 3-celled; of these, one was the vegetative nucleus and the other two, the sperms. The sperm cells occupied distal position while the vegetative nucleus remained within the microspore.

The ovule is tenuinucellate, unitegmic and anatropous. The female archesporium is one celled. Megaspore tetrads are linear (Fig. 1). Embryosac develops according to the Polygonum type and shows the usual organisation.

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1. Davis, G. L., *Systematic Embryology of Angiosperms*, John Wiley and Sons, Inc.. New York, 1966.
2. Fedortschuk, W., "Embryologische Untersuchung von *Cuscuta monogyna* Vahl. und *Cuscuta epithymum* L.," *Planta*, 1931, **14**, 94.
3. Magnus, W., "Die atypische Embryonalentwicklung der Podostemaceen," *Flora*, 1913, **105**, 275.
4. Maheswari Devi, H., "Embryology of *Jasminums* and its bearing on the composition of Cleaceae," *Acta Botanica Indica*, 1975, **3**, 52.
5. Mohrbutter, C., "Embryologische Studien an Loganiaceen," *Planta*, 1936, **26**, 64.
6. Mukkada, A. J., "Some observations on the embryology of *Dicraea stylosa* Wight.," In: *Plant Embryology, A Symposium*, CSIR, New Delhi, 1962, p. 139.
7. Narayana, L. L. and Sundari, K. T., *Embryology of Pittosporaceae—I*, 1976 (In press).
8. Shah, C. K., "Pollen development in some members of the Cyperaceae," In: *Plant Embryology, A Symposium*, CSIR, New Delhi, 1962, p. 81.
9. Sheela, R. and Narayana, L. L., "Embryology of Pittosporaceae," *Curr. Sci.*, 1966, **35**, 74.

INTERNATIONAL CONFERENCE ON METAL SCIENCES—THE EMERGING FRONTIERS, VARANASI

The Metal Sciences Division of the Indian Institute of Metals and the Department of Metallurgical Engineering of the Banaras Hindu University are jointly organising an International Conference in Varanasi from 23rd to 26th November 1977 (Wednesday to Saturday) to highlight recent researches in active frontier areas of Metal Sciences. The following topics are expected to be covered at this Conference: Solidification, Phase Transformations, Interfaces, High Resolution Microscopy, Amorphous and Magnetic Alloys, Powder Metallurgy, Composites, Thermomechanical Processing, High Temperature Deformation, Fatigue, Fracture, Corrosion, Metallurgical Thermodynamics, Diffusion, Process Simulation, Process Engineering, Metal Sciences for Society.

Details can be had from Prof. T. R. Anantharaman, Head of the Department of Metallurgical Engineering, Institute of Technology, Banaras Hindu University, Varanasi 221 005.

SHORT SCIENTIFIC NOTES

Maize Pathotype of *Corticium rolfsii* in India

During survey of Kunihar block of Solan District Himachal Pradesh, the presence of small, globose, brown to dark brown, mustard seed like sclerotia on leaf sheath and rind surface near the ground level of maize plants was observed. Isolations from single sclerotium as well as from small sterilised bits of diseased tissue taken on PDA, invariably yielded pure culture of *Sclerotium* (*Corticium*) *rolfsii*. This fungus hitherto unreported on maize was found to cause seed rot, seedling and sheath blights, rind infection and cob rot infection. Ear rotting was by far the most destructive part of the disease. The mycelium entered the kernel at the base and both embryo and endosperm were attacked, as a result of which starch-grains of the kernels were completely destroyed. The average size of the sclerotia on host plant ranged from 0·50 to 0·65 mm in diameter, while in culture sclerotia attained bigger size and ranged between 0·65 to 1·44 mm in dia.

Overwintering of the fungus occurred as dormant mycelium in old plant debris and also as sclerotia in soil. Till date, only two cereals, *viz.*, Wheat and Rice were known to harbour this fungus.

The author acknowledges thanks to the Dean, Agricultural Complex of H.P. University, for providing necessary facilities for conducting the research. He is also thankful to Dr. Mordue, Specialist and Dr. Anthony Jonston, Director, CMI, for confirming the identification (IMI 199626).

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First Record of Downy Mildew of Onion

During the past 4–5 years, severe outbreak of downy mildew of onions compelled many cultivators to abandon its cultivation in some pockets of Kashmir valley. In some areas of severe infection, the disease wiped out 60–100% of the crop.

The prevalence of downy mildew on onion has never been reported earlier from any part of India, as such, this report forms the first record of the disease.

The disease is at its peak in cool, muggy, and rainy weather. Initial symptoms are observed on the tops which die back. Primarily, the diseased parts attain a peculiar violet tint which change to greenish grey colour with age. Foliage including the stalk-bearing flowers wither and bulb becomes moist and spongy. The yield is considerably reduced.

The pathogen responsible for the disease has been identified as *Peronospora destructor* (Berk.) Casp. The sporangiophores, which are aseptate, are dichotomously branched, bearing elliptical sporangia, measuring 20–27 μ × 45–60 μ. They are thin walled and sub-hyaline. Specimen deposited with IARI, New Delhi, under reference No. HC 10–32346.

At the experimental station, Shalimar, and in the farmers' fields, it was observed, that, although the disease could not be entirely kept under check by the foliar spraying, yet 3–4 sprayings with N- Tetrachloroethyl-thio-4-cyclohexene-1, 2 dicarboximide (DIFOLATAN) gave very promising results. The above fungicide not only reduced the percentage infection of the disease but also increased the net yield of the crop.

The authors are thankful to Dr. J. N. Kapoor, Mycologist, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, for confirming our identification.

Thanks are also due to the Director of Agriculture, J and K Government, for his keen interest and facilities.

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New Host for *Colletotrichum dematium* (Pers. ex. Fr.) Grove and *C. gloeosporioides* Penz.

Colletotrichum Cda. is known to cause anthracnose, die-back, fruit rot, storage rot, leafspot, petiole rot, etc., in plants. During the course of a study[1], 84 different isolates of *Colletotrichum* were recovered from various hosts. It was found that all the isolates belonged to either of the two commonly occurring species of *Colletotrichum*, i.e., *C. dematium* and *C. gloeosporioides*. The followings are new host records for the above two species :

Colletotrichum dematium (Pers. ex. Fr.) Grove : *Alocasia macrorrhiza, Aralia spinosa, Bignonia unguiscati, Calotropis procera, Canna indica, Dalbergia sissoo, Dioscorea bulbifera, Ficus rumphii, Hamelia patens, Holoptelea integrifolia, Ixora parviflora, Livistona rotundifolia, Pongamia glabra, Rhoeo discolor, Ricinus communis.*

Colletotrichum gloeosporioides Penz : *Aralia balfourii, A. bipinnatifida, A. cordata, Aralia* sp., *Bridelia retusa, Canna* sp., *Clerodendron inermae; Cordyline terminalis, Dracaena terniflora, Ficus bengalensis, Gomphrena globosa, Ixora* sp., *Jasminum pubescence, Jatropha curcas, J. podagrica, Manihot* sp., *Ouigenia dalbergioides, Pithecolobium dulce, Plumeria rubra, Samania saman, Sansevieria roxburghiana, Vitis vinifera.*

Hosts common for both the species : *Albizzia lebbeck, Canna zebryna, Chonemorpha macrophylla, Gerbera lanuginosa, Hippeastrum johnsonii.*

The author is grateful to Dr. G. P. Agarwal, Head, Department of Post-graduate Studies and Research in Botany, University of Jabalpur, for guidance and valuable suggestions.

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1. Rajak, R. C., "Studies on the morphology and physiology of some Indian species of the genus *Colletotrichum* Cda.," *Ph.D. Thesis,* Jabalpur University, 1971, p. 222.

Cane-Rot of Ramie by *Rhizoctonia bataticola* (Taub.) Butl. in India

The author observed for the first time in Assam the cane-rot disease caused by *Rhizoctonia bataticola* (Taub.) Butl. on ramie (*Boehmeria nivea* Gaud.) which yields the strongest known vegetable fibre of commercial importance[1]. The symptoms of the disease appear as necrotic lesions on the leaves which rapidly increase and cover the entire leaf-blade. The leaves then shrink, roll, adhere to the canes and ultimately shed off. Brown, sunken, circular or elongated lesions are also common on the surface of the cane especially at the basal region. They increase in size and several such lesions coalesce and girdle the canes. When lesions form streaks along the length of the cane without girdling, the entire cane becomes shrivelled resulting in complete drying. Root system becomes weak and turns brown. A number of canes may be infected in the clump. The disease is prevalent during rainy season on mature clumps where the fungus exists as a brown mycelium and forms small black sclerotia.

The fungus was isolated in pure culture and its pathogenicity tested successfully on 30 days old ramie plants (Variety : R 1411). Breaking of canes of ramie caused by *R. solani* has been reported from Florida[2], but this is the first record of *R. bataticola* on ramie in India.

The author is grateful to Shri K. L. Ghosh, Research Officer, Ramie Research Station, Assam, for facilities and encouragement.

Ramie Research Station, T. P. MUSTAFEE.*
Indian Council of Agril.
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Sorbhog, Assam, *March* 26, 1976.

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* Present address : FCL, Maheshmati, Malda, West Bengal.

1. Ghosh, K. and Ghosh, T., *Jute Bull.,* 1971, 34 (1), 15.
2. Summers, T. E., *Diseases of Abaca Jute, Sisal and Ramie,* McGraw-Hill Book Co., Inc., 1960.

Viral Diseases of Sunflower (*Helianthus annuus* L.)—New Records from India

Besides a Mosaic virus disease of sunflower reported from Austin, Texas (Arnott and Smith[1], 1967 *b*), no viral disease so far has been reported from India. Recently during the survey of sunflower fields (1976–77), there were three distinct viral diseases, *viz.,* Yellow ring mosaic, Mosaic and Yellow spot on sunflower plants. In the first two cases the plants gave severe mosaic symptoms accompanied with stunting of plant and malformation of young leaves. In plants with Yellow ring mosaic symptoms, the flowering was either absent or the size was found to be greatly reduced.

Yellow ring mosaic and Mosaic were readily transmitted by sap as well as leaf and cleft graft. In sap transmissions, the standard sap extracted in phosphate buffer (pH 7·2, 0·02 M) with Na_2SO_3 (0·1%), using carborundum powder (600 mesh) as an abrasive, gave best results. The virus in both the cases gave chloretic local lesions on *Chenopodium amerticolor.* The Yellow spot virus could be transmitted only by grafting.

Virus Research Lab., KAMLASH C. GUPTA.
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Agra College,
Agra 282 002, *March* 30, 1977.

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1. Arnott, H. J. and Smith, K. M., *J. Ultrastructure Research,* 1967 *b,* 19, 173.

REVIEWS AND NOTICES OF BOOKS

Slow Viruses. By David H. Adams and Thomas M. Bell. (Addison-Wesley Publishing Company, Inc., Reading, Massachusetts. 01867), 1976. Pp. x + 214. Price : Hardbound $ 19.50, Paperbound $ 7.50.

In this book the authors have critically analysed several reports on slow viruses and presented their personal views on biochemical, virological and host cell-virus relationship and disease aspects of slow viruses. A slow virus is one which has a long incubation period and has both long latent phase and a slow replication rate. The quantity of virus produced in the infected organ increases progresively which is accompanied by a severe, often fatal chronic disease.

A comparison of factors controlling the macromolecular synthesis in slow and fast viruses led the authors to suggest that the key to the slow virus problem lies in the control of DNA replication. To prove this they discuss in detail the properties of Screpie virus as a typical example of slow virus. In such a situation the host cell-provirus complex exerts considerable control over subsequent events. It is suggested that the mechanism by which slow viruses produce slow virus diseases is due to lack of an effective immune or interferon response by the infected host. The lack of immune response seems to be either due to the inability of virus agent to induce the formation of new antigen in infected cells or the small quantity virus specific protein produced has not triggered the immune response.

Another slow virus, causing the subacute sclerosing panencephalitis (SSPE), is a rare complication of measles virus as it is very different from the one causing acute measles. They believe that RNA measles virus is altered to a DNA form by the process of reverse transcription giving a slow virus. It is proposed that the slow viral nature is based on the DNA content and the limited ability to take over and direct the metabolic activity of its host cells and as a consequence the viral genome gets integrated into the host genome. From such an integrated form, the transcription of viral *m*RNA and its translation into protein is frequently incomplete. They therefore conclude that the DNA would be a more suitable core for a slow virus than RNA.

The authors discuss the incidence of slow virus infection and the clinical aspects of human diseases caused by slow viruses and present their views on why the central nervous system might be particularly affected by slow virus diseases. The book ends with several useful references and subject index. This book should interest the medical virologists and other research workers in virology.

A. ANTONY.

Pest Control in Rice. (*PANS Manual No. 3*—Second Edition, completely revised) (Centre for Overseas Pest Research, London), 197[illegible]. Pp. 295. Price : £ 2.25, postage extra.

Rice is the staple food of millions of people, particularly in the tropical countries and the rice plant is attacked by a very large complex of insects, mites and other injurious animals besides a wide range of pathogens. Some of these destructive agencies cause heavy losses in yield. Weeds also greatly reduce rice production in many parts of the world. The handbook under review gives up-to-date information on all aspects of crop protection in rice cultivation. The introduction gives a brief account of the distribution, botany, classification and agronomy of rice. The following sections cover, respectively, weeds, diseases (including physiological disorders), nematodes, molluscs, Crustacea, insects and mites, birds and rodents. There is also a section dealing with storage problems. Each destructive organism is briefly described, its life-history summarised, damage symptoms are given and control measures suggested. The control measures are mostly either cultural or chemical. Pesticide application equipment and techniques are succinctly explained in a separate section. At the end of each section there is a select bibliography for further reading. The manual includes 124 text-figures, 8 colour plates and 23 tables, which add to the practical value of the publication. An Appendix gives checklists of diseases, insects, nematodes and pesticides. Common names of organisms listed and some synonyms and misidentifications are also mentioned. There is a combined subject and species index at the end of the manual.

T. SANKARAN.

6394/77. Published by S. R. S. Sastry, Manager, Current Science Association, Bangalore-560006
and Printed at The Bangalore Press, Bangalore-560018.

L-ASPARAGINASE ACTIVITY IN MARINE SEDIMENTS

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ABSTRACT

L-asparaginase activity and L-asparaginase producing bacteria were estimated in sediment samples collected from three different biotopes—marine, estuarine and the mangroves. In the mangrove areas, the rhizosphere soils of *Rhizophora* and *Avicennia* showed maximum activities. The enzyme showed two pH optima, one at 6·2 and the other at 8·7. The Km value was found to be optimum and further increase, inhibited the enzyme activity. Dialysed samples exhibited slightly higher activity and sodium chloride did not show any effect on the activity even at a concentration of 10%. The occurrence of enteric bacteria in these sediments is also reported. Various factors governing the asparaginase activity are discussed.

INTRODUCTION

BROOME[1] showed that L-asparaginase (L-asparagine amidohydrolase EC 3·5·1·1) was responsible for antitumour activity of guinea-pig serum[2] and further studies showed that this enzyme can effectively be employed in the treatment of acute leukaemia[3,4]. Since then many reported the occurrence and activities of this enzyme in various bacteria[5], yeasts[6], fungi[7], and atinomycetes[8] but it was found that not all the asparaginases from the different sources did exhibit antitumour and anii-leukaemic properties. It is therefore imperative to screen for new strains from different ecological niches for asparaginases having anti-tumour and anti-leukaemic properties and with this in mind, studies on asparaginase from marine environments were initiated in this laboratory. The present report details the occurrence and activity of asparaginase in marine sediments of Porto Novo.

MATERIALS AND METHODS

The sediment samples were collected from ten stations near Parangipettai (Lat. 11° 29′ N; Long. 79° 49′ E) representing different biotopes (Table I) with the help of a Peterson grab. Samples for enzyme analysis were prepared as described earlier[9]. The salinity of the overlying waters was determined with the aid of a salinometer and the pH with a Philips pH meter.

TABLE I

L-asparaginase activities in different sediments

Section No. and Description	Sediment type	Depth (M)	Salinity of the overlying water (%)	Temperature of the overlying water (° C)	pH	L-asparaginase activity (μm NH$_3$/g)	Enteric Bacterial population	
							NLF (×10³)	LF (×10²)
1. Sea	Silt	20·00	33·19	25·5	8·0	0·48	—**	—
2. Sea	Silt	14·00	32·83	26·0	8·1	0·16	—	—
3. Mouth of Vellar estuary	Silt	2·00	31·57	26·5	7·8	1·44	10·09	1·31
4. Estuary	Clay	2·00	31·28	26·5	8·0	0·96	9·93	1·49
5. Backwater	Silt	1·20	32·11	26·5	8·0	N.D.*	—	—
6. Mangrove	Clay	0·63	33·73	27·3	8·1	4·80	16·66	22·20
7. Mangrove	Clay	1·10	33·73	27·0	8·0	6·76	—	—
8. Mangrove	Clay	1·14	25·69	30·0	7·5	4·32	18·11	18·12
9. Rhizosphere soil *Avicennia officinalis*	Clay	0·20	31·03	33·0	7·3	5·12	—	—
10. Rhizosphere soil of *Rhizophore mucronata*	Clay	0·30	28·55	32·5	7·6	8·96	—	—

N.D.* Not detectable: ** Not estimated

The enzyme assay was carried out as follows. To 5 g of air dried powdered sediment, 15 ml of 0·1 M Tris-HCl buffer (pH 8·7) containing 0·2 M asparagine and 0·5 ml toluene were added before the mixture was incubated at 40° C for 24 hours. After incubation, 5 ml of N KCl was added and the mixture was shaken to release the adsorbed ammonia into the solution. The contents were filtered through Whatman No. 1 filter paper and the ammonia content in the filtrate was estimated by the Conway microdiffusion method. The filtrate (1·5 ml) was placed in the outer well and 0·6 ml of 1% boric acid was placed in the central well. To the outer well, 1·5 ml of saturated K_2CO_3 was added, the dishes were covered and incubated at 40° C for 3 hrs. The ammonia absorbed in boric acid was determined by Nesslerization and the extinction was measured at 450 mμ in a Spectronic-20 colorimeter. Suitable controls were maintained throughout the period of investigation.

Total enteric bacteria in sediment samples were estimated by plating with MacConkey agar. The lactose-fermentors (L.F.) and non-lactose fermentors (N.L.F.) were calculated per gram sediment on dry weight basis.

For the enzyme kinetic studies, sample collected at Station 8 was employed.

RESULTS AND DISCUSSION

Asparaginases can be obtained from a variety of sources but the enzyme, obtained from *E. coli*[9], *Erwinia caratovora*[10], *Serratia maceescens*[11], *Mycobacterium tuberculosis*[12], *Azotobacter vinelandii*[13], Guinea-pig serum[14,15] and the serum from a variety of other members of the super-family Caviodea[16], has shown anti-tumour activity. Except for one report on the presence of this enzyme in two marine bacteria *Aeromonas hydrophilla* and *Aeromonas liquifaciens*[5] nothing is known about the other sources from bacteria, fungi and antinomycetes from marine environment. Asparaginase activity could be traced positively in all marine estuarine and mangrove sediments in the present study.

After Drobnik's[17] report Mouraret[18] made an extensive study of asparaginase activity in soils. However higher activities could be recorded in mangrove regions and in the rhizosphere soils of *Rhizophora mucronata* and *Avicennia officinalis*. In general clayey sediments always exhibited higher activities than the silty ones. Asparaginase activity could be recorded in all samples, except at Station 5, irrespective of variations in salinity, temperature and pH.

In order to ascertain the effect of time of incubation on the enzyme activity, the activity was measured at 6 hr. intervals upto 48 hr at 40° C. The results indicated that even a 24 hr. incubation period resulted in as much as 93% of the total activity (Fig. 1).

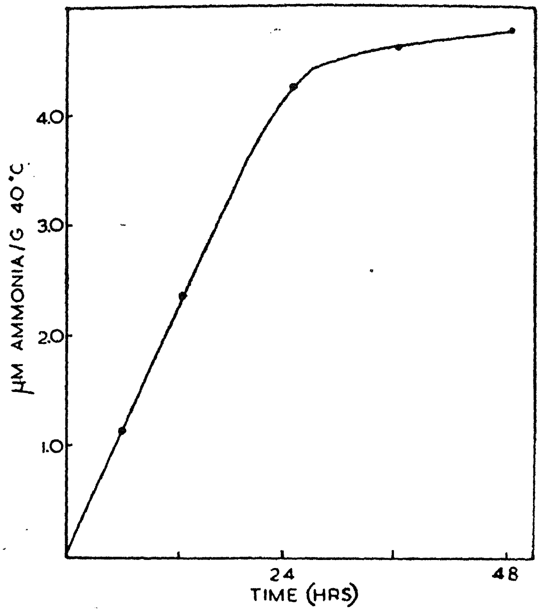

FIG. 1

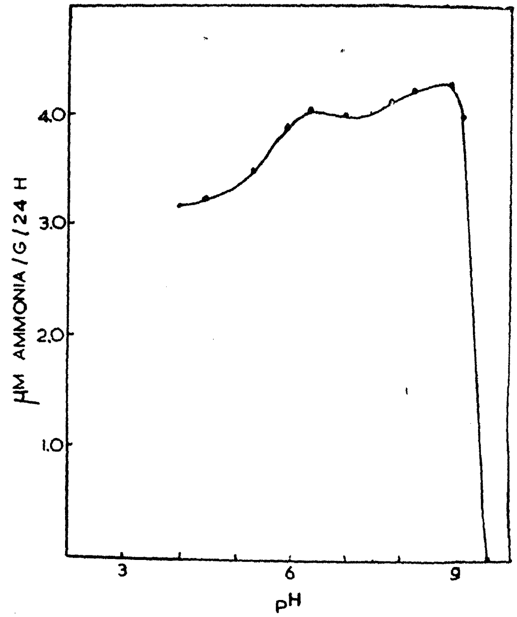

FIG. 2

Asparaginase activity as a function of pH at 40° C is shown in Fig. 2. The buffers used were (*i*) 0·1 M Tris-HCl (pH 7·2–8·9); (*ii*) 0·1 M Citrate buffer (pH 4·0–6·2); and (*iii*) 0·1 M Glycine–NaOH (pH 9·6–10·0). The activity curve showed two pH maxima, one at 6·2 and the other at 8·7. Possibly the marine sediments contain asparaginases from different sources; from extracellular secretions of microbial cells or from intracellular enzymes released after the death of the cells, or again the observed peak at pH 6·2 may be due to the buffer effect. It has been reported earlier that most of the enterobacterial asparaginases have pH–optima around 8·5[5]. The sediment samples, in the present study harboured members of *Enterobacteriaceae* (Table I), and selected isolates of *Escherichia coli* produced asparaginase at an optimum pH of 8·7. Thus asparaginase activity measured at pH 8·7 in marine sediments may be due to *E. coli,* though the possibility of similar pH–optima for other microbial asparaginases cannot be ruled out. Asparaginase of *Streptomyces griseus* has a pH-optimum of 8·5[8]. It is interesting to note that the marine sediments also harbour members of *Streptomycetaceae*[19,20].

The effect of substrate concentration on the activity was studied by incubating the sediment samples with varying concentrations of asparagine. Maximum activity was reached at a concentration of 0·233 M and no inhibition occurred at higher concentrations (Fig. 3). Similarly, Mouraret[18] and Drobnik[17] used a substrate concentration of 2·5% and 3% respectively to study the asparaginase in soils. The Km value was calculated by means of Lineweaver-Burk plot (Fig. 4) and it was found to be $2 \cdot 17 \times 10^{-3}$ M. Similar Km values for asparaginases have already been reported for certain bacteria (Table II) and this indicates that marine sediments may also harbour similar asparaginase producers.

TABLE II

K_m values of different L-asparaginases

Source	K_m (M)
Guinea-pig serum[15]	$7 \cdot 2 \times 10^{-5}$
Escherichia coli[26]	$1 \cdot 25 \times 10^{-5}$
Erwinia aroideae[27]	3×10^{-3}
Bacillus coagulans[28]	$4 \cdot 7 \times 10^{-3}$
Fusarium tricinctum[29]	$5 \cdot 2 \times 10^{-5}$
Proteus vulgaris[30]	$2 \cdot 6 \times 10^{-5}$
Vibbrio succinogenes (anaerobic)[31]	$1 \cdot 7 \times 10^{-5}$
Candida utilis[6]	$1 \cdot 1 \times 10^{-4}$
Hansenula jadini[6]	$1 \cdot 3 \times 10^{-4}$
Rhodotorula rubra[6]	$5 \cdot 5 \times 10^{-5}$
Marine sediment (present study)	$2 \cdot 17 \times 10^{-3}$

The effect of temperature on the stability of the enzyme is given in Fig. 5. Sediment samples *plus* buffer were incubated at 10, 20, 30, 40, 50,

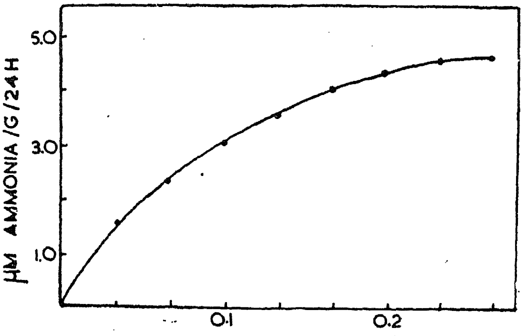

FIG. 3

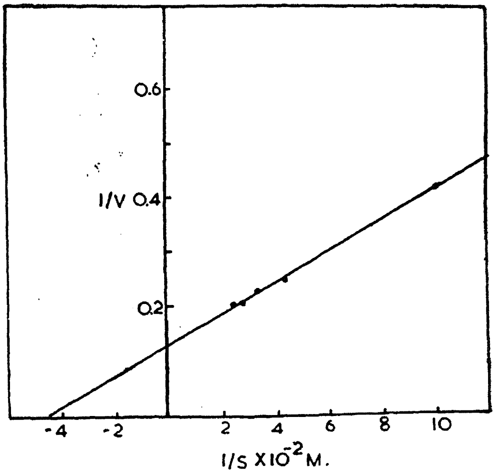

FIG. 4

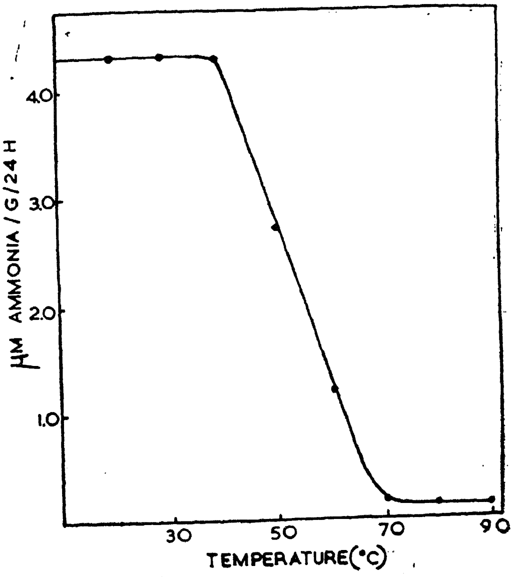

FIG. 5

60, 70, 80, 90° C for one hour. After incubation the substrate solution was added to each sample and the incubation continued at 40° C for 24 hrs. The enzyme remained unaffected upto 40° C but further increase in temperature resulted in loss of activity. Maximum activity could be recorded at 40° C. At 70° C almost 97% of the activity was lost and at 90° C, total loss of activity was recorded. Rotini[21] while examining urease activity at higher temperatures (58° C), registered a high activity and suggested the contribution of urease by lysis of micro-organisms. He observed that the increase in activity was more at this temperature in the presence of toluene. When the heat stability of the enzyme was studied it was found to retain its total activity only up to 40° C and at 70° C nearly 97% of the total activity was lost. Galstyan[22], while examining the effect of temperature on the inactivation of soil enzymes, reported that the inactivation of enzyme in soil occurs at 10° C higher than that in solution[23]. This indicated that when the heat stability of the enzyme was studied, the effect of temperature would be only on the free enzymes of the sediment and since the sediments were exposed to different temperatures for only one hour, there is not much contribution by lysis of the microbial cells. Drobnik[17] and Mouraret[18] also employed higher temperatures (42° C and 49° C respectively) for estimating asparaginase activity in soils.

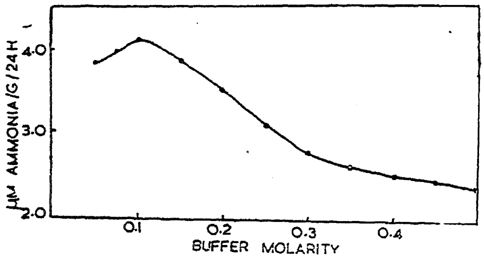

FIG. 6

The effect of buffer molarity on enzyme activity was also studied (Fig. 6). Tris-HCl buffer (pH 8·7) was employed at various concentrations to study its influence on enzyme activity. Maximum activity could be observed at 0·1 M and any further increase in molarity inhibited the enzyme activity.

Effect of sodium chloride on asparaginase activity is shown in Fig. 7. The sediment samples were dialysed against distilled water at 4° C for 24 hrs and the samples were air dried at room temperature (28 ± 2° C) in the laboratory. The dried samples were again powdered and used for the enzyme assay. To the enzyme substrate mixture sodium chloride was added at varying concentrations upto 10%. The results indicate that NaCl even at a concentration of 10% did not have any effect on enzymes activity. This suggests that the asparaginase activity is not affected by changes in salinity under natural conditions. Dialysed samples always showed slightly higher activity than the undialysed ones. This may be due to the removal of some inhibitory factors along with sodium chloride during dialysis. It may be mentioned here that phosphates and nitrates, at higher concentrations, are known to inhibit soil asparaginases[18].

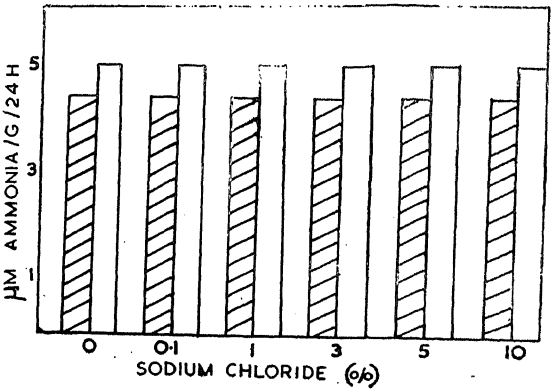

FIG. 7

Previous reports on phosphatase[24], arylsulfatase[9] and nuclease[25] activities in marine sediments indicate the possible role of free enzymes in regeneration of nutrients in marine environment. Asparaginase may also have a similar role, at least to a limited extent, by releasing ammonium for nitrification. In addition, the partial purification and the antitumour property of the enzyme from one culture (MEB-130) has been completed and the results will be published elsewhere.

ACKNOWLEDGEMENTS

We are thankful to Dr. J. Skujins, Department of Biology, Utah State University, U.S.A., for his comments and help. The financial support from the University Grants Commission, New Delhi, India, is gratefully acknowledged.

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1. Broome, J. D., *Nature*, 1961, 19, 1114.
2. Kidd, J. G., *J. Exptl. Med.*, 1953, 98, 565.
3. Mashburn, L. T. and Wriston, J. C., *Arch. Biochem. Biophys.*, 1964, 105, 451.
4. Hill, J. M., Ellenloeb, Ayten Macleilen, Khan, A. and Hill, N. O., *Symposium International Sur La L-Asparaginase*, Colloques Internationaux, C.N.R.S., Paris, 1970, p. 273.
5. Wade, H. E., Robinson, H. K. and Phillips, B. W., *J. Gen. Microbiol.*, 1971, 69, 299.
6. Imada, A., Nakahama, K., Igarasi, S. and Takeda, J., *Res. Lab.*, 1972, 31, 460.

7. Nakahama, K., Imada, A. and Igarasi, S., *J. Gen. Microbiol.*, 1973, 75, 269.
8. Dejong, P. J., *Appl. Microbiol.*, 1972, 23, 1163.
9. Chandramohan, D., Devendran, K. and Natarajan, R., *Mar. Biol.*, 1974, 27, 89.
10. Wade, H. E., Elsworth, R., Herbert, D. Kippe, J. and Sargeant, K., *Lancet*, 1968, 11, 776.
11. Rowley, B. and Wriston, J. C., *Biochem. Biophys. Res. Commun.*, 1967, 28, 160.
12. Subba Reddy, V. V., Jayaram, H. N., Sirsi, M. and Ramakrishnan, T., *Arch. Biochem. Biophys.*, 1969, 132, 262.
13. Gaffar, S. A. and Sethna, Y. I., *Curr. Sci.*, 1975, 44, 727.
14. Clementi, A., *Arch. Intern. Physiol.*, 1922, 19, 369.
15. Yellin, T. O. and Wriston, J. C., *Biochemistry*, 1966, 5, 1605.
16. Old, L. J., Boyse, E. A., Campbell, H. A. and Daria, G. M., *Nature*, 1963, 198, 801.
17. Drobnik, J., *Mikrobiol.*, 1956, 1, 47.
18. Mouraret, M., *Office de la Pechaercia Scientifique et Technique outre Mer. Paris*, 1965.
19. Paulraj, S., *Studies on Certain Aspects of Marine Streptomycetes*, M.Sc. Thesis, Annamalai Univ., India, 1973.
20. Chandramohan, D., Ramu, S. and Natarajan, R., *Curr. Sci.*, 1972, 41, 245.
21. Rotini, O. T., *Ann. Labor. Ferm., "Spallanzani"*, 1935, 3, 173.
22. Galstyan, A.. *Sh. Dokl. Akad. Nauk. SSSR*, 1965, 40, 177.
23. Skujins, J., *Soil Biochemistry*, Mclaren, A. D. and Peterson, G. H., New York, 1967, p. 371.
24. Ayyakkannu, K. and Chandramohan, D., *Mar. Biol.*, 1971, 11, 20.
25. Maeda, M. and Taga, J., *Ibid.*, 1973, 20, 58.
26. Broome, J. D., *Trans. N.Y. Acad. Sci.*, 1968, 30, 690.
27. Peterson, R. E. and Ciegler, A., *Appl. Microbiol.*, 1969, 18, 64.
28. Law, A. S. and Wriston, J. C., *Arch. Biochem. Biophys.*, 1971, 147, 744.
29. Raymond, W. Scheetz, Helen, A. Wheelan, and John, C. Wriston, *Ibid.*, 1971, 142, 184.
30. Tosa, T., Sano, R. R., Yamamoto, K., Nakamura, M. and Chibata, I., *Biochemistry*, 1972, 11, 217.
31. David Kafkewitz, and David Goodman, *Appl. Microbiol.*, 1974, 27, 206.

PREPARATION AND NMR STUDIES OF BENZO (*h*) QUINOLINE DERIVATIVES

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MONO nitration of 2, 4-dimethyl benzo (*h*) quinoline has been achieved by the use of nitric acid. NMR studies have served to establish the structures of the two products as the 9 and 7-nitro, 2, 4 dimethyl benzo (*h*) quinolins. The NMR spectra of benzo (*h*) quinoline as reported by E. Vender Donckt[1] is very complicated. The spectrum could be simplified by preparing its suitable derivatives. Although many of the reactions of benzo (*h*) quinoline and its derivatives have been thoroughly studied[2], nitration has received only limited attention. Baltrop and McPhee[3] recently investigated the nitration of 1-azaphenanthrene by Haid's[4] directions and separated the isomers on an alumina column monitored by ultraviolet light. These authors have shown that in 1-azaphenanthrene, electrophilic substitution takes place preferentially at the 9 and 7 positions. There is no report of an electrophilic reaction of 1-azaphenanthrene containing a functional group in the heteroaromatic ring. This investigation was undertaken in order to develop a practical method for the preparation of these compounds.

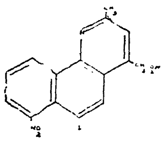
Results and Discussion

Although several workers have claimed to have isolated 2, 4-dimethyl benzo (*h*) quinoline, no direct evidence for its formation has been obtained. Melting point also varies as reported in the literature. Vasserman[5] reported a melting point of 44°, as did Combes[6] and Reed[7]. Johnson and Mathews[8], reporting 51·5–53°, agreed with Van Braun[9] at 52° but Scherk and Baily[10] gave the melting point as 55–56°.

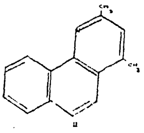
Definite evidence for its structure was, therefore, needed. The NMR spectra were consistent with all the intermediates and end product. The H–10 signal in the NMR spectrum at 60 M/C of 2, 4-dimethyl benzo (*h*) quinoline arising from the ring protons is very little informative due to its complexity. This is caused by the coincidence of most of the chemical shifts resulting in strongly overlapping signals. H–3 proton and 2 and 4 methyl groups on ring containing hetero atom are easily detectable. The difficulties in the interpretation of the very complex pattern of the low field H–10 signal could be alleviated by a further increase of the spectrometer frequency, because this leads to a corresponding increase of the chemical shift differences and hence to a simplification of the analysis of spectra.

An important progress in this connection has been achieved by the introduction of high resolution 100 MHz NMR spectrometer using magnets of high magnetic field strength. A comparison of the 60 and 100 MHz spectra shows that the former already reveals all spectral subtleties of the ring containing nitrogen atom whereas the low field signal of H–10 is not very informative. All other protons give rise to the complex patterns. The situation is completely different in the second spectrum presenting the H-10 proton at 100 MHz. Here, this part consists of 16 lines showing ortho, meta, para and long range coupling.

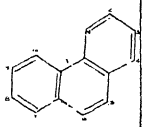
The successful nitration of 1-azaphenanthrene with nitric acid prompted us to investigate this method for the benzo (*h*) quinoline system. 2, 4-dimethyl benzo (*h*) quinoline when nitrated gave compounds I and II which are yellow crystalline solids and have melting points of 174° and 194° C respectively. The mass spectra and quantitative analysis of both substances support the correctness of the molecular formula ($C_{15}H_{12}N_2O_2$ assigned for a mononitro 2 4-dimethyl benzo (*h*) quinoline.



7-NITRO 2 4 DIMETHYL BENZO (h) QUINOLINE MELTING POINT 174°C



9-NITRO-2,4-DIMETHYL BENZO (h) QUINOLINE MELTING POINT 194°C



BENZO (h) QUINOLINE

The assignment of the positions of the nitro group in I and II presents a problem. In the series pyridine < quinoline < isoquinoline < acridine < benzo (*h*) quinoline, the ease of nitration increases significantly. Pyridine gives 22% yield of 3-nitro pyridine when a solution of pyridine in 100% sulphuric acid reacts with potassium nitrate and nitric acid at 300° C. This is contrasted with the quantitative yield of mononitro benzo (*h*) quinoline obtained when benzo (*h*) quinoline nitrate was dissolved in conc. sulphuric acid. The increasing reactivity of the benzo and dibenzo pyridines can be attributed to the decreasing effect of the hetero nitrogen atom on the more distant benzenoid ring. This leads to an increased polarizability at the C atom being nitrated. A comprehensive review of the experimental results and theoretical implications of aza-aromatic nitration has been prepared by Schofield[11].

Since it is extremely unlikely that a heterocyclic ring would be nitrated under the conditions used, the logical positions are C (7) and C (9). Collateral evidence in support of this is provided (*a*) by the fact that phenanthrene, through steric hindrance, is resistant to substitution at C (4) which corresponds to (C10) in benzo (*h*) quinoline and (*b*) by Longuet-Higgins and Coulson's calculations (*J. Chem. Soc.*, 1949, p. 977). The authors computed the electron density at the various C atoms of benzo (*h*) quinoline and showed that the highest electron densities are located at C (9) and C (7). It may be objected that the entity existing in the nitration is the salt of 2, 4-dimethyl benzo (*h*) quinoline whereas the calculations of electron density are given only for the uncharged form of benzo (*h*) quinoline; however, the nitration products of 2, 4-dimethyl benzo (*h*) quinoline in the order of decreasing abundance are the 9- and 7-nitro, 2, 4-dimethyl benzo (*h*) quinoline. The mononitro structures are further confirmed by their NMR spectra. The NMR data of the 2, 4-dimethyl, 7 nitro 2, 4-dimethyl and 9 nitro 2, 4-dimethyl benzo (*h*) quinolines are shown in Table I.

TABLE I

NMR data of benzo (h) *quinoline derivatives*

Compound	H-3	H-5	H-6	H-7	H-8	H-9	H-10	$2-CH_3$	$4-CH_3$
2, 4-dimethyl benzo (*h*) quinoline	$\tau = 2·9$						$\tau = ·69$	$\tau=7·32$	$\tau = 7·5$
7-nitro-2, 4-dimethyl benzo (*h*) quinoline	$\tau=2·72$	$\tau = 1·98$	$\tau=1·62$	..	$\tau =1·72$	$\tau=2·27$	$\tau=0·26$	$\tau=7·26$	$\tau=7·33$
(c/s)	$J_{5,6}=9·5$	$J_{9,10}=8·2$	$J_{8,10}=1·4$	$J_{8,9}=7·8$			long range coupling = ·7		
9-nitro-2, 4-dimethyl benzo (*h*) quinoline	$\tau=2·72$	$\tau=1·95$	$\tau=2·24$	$\tau=2·09$	$\tau=1·55$		$\tau=-·15$	$\tau=7·25$	$\tau=7·32$
(c/s)	$J_{8,10}=1·4$	$J_{7,8}=8$	$J_{5,6}=9·5$	$J_{7,10}=0·4$			long range coupling = ·7		

Consideration of spectra of these compounds permitted the assignments shown and the basis for making the assignments is discussed below.

For the aromatic protons of the 9-nitro derivative the least deshielded one. H–7 appears as a broadened doublet, predicted by a first order treatment for the ortho coupling to H–8 and para coupling to H–10. H–8 and H–10 are also deshielded by the nitro group. H–10 appears further downfield as compared to its position in 2, 4-dimethyl benzo (*h*) quinoline.

The positions of the aromatic proton signals in the 7-nitro isomer reflect the deshielding influence of the nitro group as expected H–8 > H–10 > H–9. The H–8 signal shows a major ortho coupling with H–9 broadened by meta coupling with H–10.

Of major importance is the evidence provided by the C (5) and C (6) protons which give a doublet and is consistent with the nitro group being in the 7 and 9 positions.

Experimental

4-(1-*naphthylimino-)-pentanone*-2 *and* 2, 4-*dimethyl benzo* (h) *quinoline* were prepared according to the method given in the literature[8].

Nitration of 2, 4-*dimethyl benzo* (h) *quinoline*

2, 4-dimethyl benzo (*h*) quinoline (1 g) was dissolved in glacial acetic acid (10 ml) and nitric acid (2, 1 ml) was added dropwise. The white precipitate formed was filtered off, washed with a little alcohol and dried. It was treated with conc. sulphuric acid (10 ml) and heated on a steam bath for one hour. The contents were poured on to crushed ice (200 g) and made alkaline. The residue (2 g, m.p. 152°) was removed by filtration. The benzene chloroform extract (1:1) of the residue was chromatographed on an alumina column and developed with benzene. The benzene eluates on evaporation yielded yellow solids, which on crystallisation from ethanol gave pure specimens of two isomeric nitro 2, 4-dimethyl benzo (*h*) quinoline. The higher melting isomer (0·8 g, m.p. 194°) was found to be 9-nitro-2, 4-dimethyl benzo (*h*) quinoline.

Yield (65·7%.)

Analysis calculated for $C_{15}H_{12}N_2O_2$

C = 71·41; H = 4·80; N = 11·11.

Found C = 71·14; H = 4·83; N = 10·90.

The lower melting isomer (·4 g, m.p. 174°) was found to be 7-nitro, 2, 4-dimethyl benzo (*h*) quinoline

Yield = 32·8%

Calculated for $C_{15}H_{12}N_2O_2$

C = 71·41; H = 4·80; N = 11·11.

Found

C = 71·13; H = 4·85; N = 10·85.

Total Yield = 1·2 g (98·5%).

Mass spectra

9-nitro, 2, 4-dimethyl benzo (*h*) quinoline

m/e (relative intensity)

206 (100), 252 (50), 194 (30), 191 (15%).

I.R. (Nujol mull), 710 (M), 740 (M), 780 (S), 840 (M). 960 (M), 1340 (S), 1370 (S), 1580 (S), 1610 (M) cm^{-1}.

7-nitro, 2, 4-dimethyl benzo (*h*) quinoline

m/e (relative intensity)

194 (100), 252 (50), 206 (25), 222 (15).

I.R. (Nujol mull) 715 (M), 750 (S), 880 (M), 1350 (S) 1400 (S), 1466 (S), 1484 (M), 1600 (S), 1605 (M) cm^{-1}.

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1. Vender Donckt, E., *Tetrahedron*, 1964, **20**, 1495.
2. Allen, C. F. H., *The Chemistry of Heterocylic Compounds*, Interscience, New York, 1958, pp. 172 and 185.
3. Baltrop and McPhee, *J. Chem. Soc.*, 1952, p. 638.
4. Haid, *Monatsh*, 1906, **27**, 315.
5. Vasserman, Trudy Dnepropetrov. Khim-Tekhmol Inst. in F. E. Dzerzhins-Kogo, *Kafredra Org. Khim*, 1938, 68; *Khim Refrar. Zhur*, 1939, **2**, 56; *Chem. Abstracts*, 1940, **34**, 1020.
6. Combes, *Compt. rend.*, 1888, **106**, 1536.
7. Reed, *Ji Prakt. Chem.*, 1887, **35** (2), 298.
8. Johnson and Mathews, *J. Am. Chem. Soc.*, 1944, **66**, 210.
9. Van Braun and Wolff, *Ber.*, 1922, **55 B**, 3675.
10. Schenck and Bailey, *J. Am. Chem. Soc.*, 1941, **63**, 2331.
11. Schofield, K., *Quart. Revs.* (London), 1950, **4** 382.

COPPER (II), NICKEL (II) AND COBALT (II) COMPLEXES OF ACYLHYDRAZINE SCHIFF BASES

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ABSTRACT

Cu^{II}, Ni^{II} and Co^{II} complexes of Schiff bases, derived from O-amino benzoylhydrazine, salicylaldehyde and benzaldehyde (1:1:1) and O-amino benzoylhydrazine and O-hydroxyacetophenone (1:2) were prepared from aqueous and ethanolic media and characterized by analytical, spectroscopic and magnetic data. The complexes are coloured, insoluble in common organic solvents, melt or decompose above 200° and adopt planar or octahedral stereochemistry.

INTRODUCTION

SCHIFF bases derived from acylhydrazines act as multidentate ligands and react in their keto-enol forms[1-5]. In view of this we presently report Cu^{II}, Ni^{II} and Co^{II} complexes with a Schiff base (H_2J) derived from O-aminobenzoyl hydrazine and O-hydroxyacetophenone (1 : 2) and a mixed Schiff base (HL) from O-aminobenzoylhydrazine, salicylaldehyde and benzaldehyde (1 : 1 : 1) which is different from the disalicylaldimine reported earlier[2].

EXPERIMENTAL

Preparation of the Ligands

The ligand H_2J and HL were prepared by reacting O-aminobenzoylhydrazide[6] (m.p. 120°, Lit. 121°) and O-hydroxyacetophenone (1 : 2) and salicylaldehyde *plus* benzaldehyde (1 : 1 : 1) respectively. The products were purified by recrystallization. (H_2J, m.p. 191. Found C, 70·41 ; H, 5·32 ; N, 10·43 ; $C_{23}H_{21}N_3O_3$ Reqd. C, 71·32 ; H, 5·42 ; N, 10·85%) (HL, m.p. 200°. Found : C, 74·10 ; H, 5·13 ; N, 12·02 ; $C_{21}H_{17}N_3O_3$ Reqd. C, 73·47 ; H, 4·96, N; 12·24%).

Preparation of the Complexes

The metal salts of BDH, AR grade were used for preparation of the complexes which were obtained by refluxing the ligands with the metal salts in 1 : 1 molar ratio either in alcoholic or aqueous medium keeping metal salts slightly in excess. The products were washed with either ethanol or water and dried over anhydrous calcium chloride.

Analysis

Metal part in the complexes was analyzed by following literature procedures after destroying the organic moiety. The ligands were estimated volumetrically[2], while chloride was determined gravimetrically and nitrogen microanalytically using a Coleman nitrogen analyzer. Analytical and some general characteristics of the complexes are given in Table I.

TABLE I

Analytical, electronic and magnetic moment data of Cu^{II}, Ni^{II} and Co^{II} complexes of Acylhydrazine Schiff bases H_2J and HL

Sl. No.	Complex and colour	Dec. pt.	Analysis (%) Metal	Nitrogen	Ligand	Chloride	Magnetic moment μ_{eff} B.M.	Electroinc bards λ_{max} nm
(*a*)	Cu (HJ) Cl Green	>250	12·6 (13·1)	9·0 (8·6)	80·4 (79·6)	6·9 (7·3)	1·9	255, 325, 342, 360, 380, 410, 625.
(*b*)	Gu $(HJ)_2$ Green	>250	7·3 (7·6)	9·8 (10·0)	91·32 (92·4)	Nil	1·2	225, 255, 300, 395, 625.
(*c*)	NiJ Red	>250	13·7 13·2	9·8 9·4	87·2 86·7	Nil	diamagnetic	250, 285, 356, 388.
(*d*)	CuLCl Green	200 m	14·0 (14·4)	9·3 (9·5)	76·9 (77·6)	7·9 (8·0)	2·0	210, 273, 290, 405, 685.
(*e*)	$NiLCl\cdot H_2O$ Green	>250	13·6 (13·0)	9·5 (9·2)	76·1 (75·2)	8·0 (7·8)	3·1	250, 295, 310, 430, 950.
(*f*)	$CoLCl.H_2O$ Reddish Brown	>250	12·8 (13·0)	8·8 (9·2)	75·8 (75·2)	7·5 (7·8)	4·7	250, 290, 435, 565, 1175.

m = melts.

I.r. and electronic spectra, in nujol, were recorded on a Perkin–Elmer 621 and a Cary 14 respectively. Magnetic measurements were carried out on a Faraday balance using $Hg[Co(NCS)_4]$ as the calibrant. Diamagnetic corrections were applied using Pascal's constants. Some important, i.r. bands are given in Table II.

TABLE II

Some important i.r. bands of H_2J, HL and their Cu^{II}, Ni^{II}, Co^{II} complexes

Sl. No.	Complexes	ν(OH) + ν(NH)	ν(C=O)	ν(C=N)	Amide II + ν(C—O) Phenyl	Amide III
	H_2J	3490 3380 3220	1625	1610	1584 1548	1365
(*a*)	Cu (HJ) Cl	3315 3125 3100	1610	1610	1584 1540	1400 1373
(*b*)	$Cu (HJ)_2$	3490 3375 3210	1623	1605	1569 1545	1375
(*c*)	NiJ	3260 3195 3070	..	1602 1590	1570 1525	1385 1365 1355
	HL	3376 3316	1642	1614 1600	1542	1371
(*d*)	CuLCl	3458 3436 3216 3084	1616	1604	1570	1392
(*e*)	$NiLCl{\cdot}H_2O$	3360 3240	1617	1600	1570 1544	1390
(*f*)	$CoLCl{\cdot}H_2O$	3250 3066 2974	1617	1596	1667 1542	1390

RESULTS AND DISCUSSION

Cu^{II} forms 1 : 1 complexes with both the ligands in ethanol but 1 : 2 $Cu(HJ)_2$ in aqueous medium. Ni^{II} and Co^{II} yield 1 : 1 complexes in ethanol but fail to react in aqueous medium.

The complexes prepared in the ethanolic medium carry one chloride ion. The complexes are coloured and insoluble in common organic solvents and decompose above 250° except the Cu^{II} complex (d) which melts at 200°. A broad band in 3600–3400 cm^{-1} region indicates the presence of water molecules in some of the complexes.

Electronic absorption bands of H_2J at 222, 245, 295 and 375 nm and those of HL at 238, 305 and 325 nm, considerably shift towards red in the metal complexes due to metal-ligand bonding. The complexes (*a*, *b*, *d*) absorb at 625, 625 and 685 nm respectively like other planar complexes[7]. The colour, absorption bands and magnetic data of Ni^{II} complexes (*c* and *e*) correspond to planar and octahedral stereochemistry respectively like salicylaldimine complexes[1]. Similarly Co^{II} complex (*f*) appears to have mixed planar and octahedral stereochemistry in solid state.

Broad, i.r., bands centred at 3490, 3380, 3220 cm^{-1} in H_2J and 3370, 3316 cm^{-1} in HL due primarily to ν (OH) + ν (NH) show the presence of strong intramolecular hydrogen bonding. The presence of these bands in the complexes suggest the presence of H-bonded residues in complexes. Bonding through $>$C=O is, however, evident from the negative shift in ν (C=O). Similar, but small, negative shift in ν (C=N) may be due to coordination of one $>$C=N group. Amide II (1550 cm^{-1}) bands in the ligands split and show positive as well as negative shifts confirming, indirectly, the coordination of $>$C=O group. ν (C—O) (1250 cm^{-1}) also shifts to higher frequency on chelation.

In NiJ (*c*), however, ν (C=O) (1625 cm^{-1}) of ligand is replaced by a doublet around 1600 cm^{-1} indicating the absence of $>$C=O group due to enolization. Bonding through NCO⁻ groups[8] is evident from a strong band around 1525 cm⁻¹.

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1. Narang, K. K. and Aggarwal, A., *Inorg. Chim. Acta*, 1974, 9, 137; *Indian J. Chem.*, 1975, 13, 1072.
2. Narang, K. K. and Lal, R. A., *Indian J. Chem.*, 1976, 14 A, 442.
3. Biradar, N. S. and Havinale, R. R., *Inorg. Chim. Acta*, 1976, 17, 157.
4. Iskander, M. F., El Sayed, L. and Lasheen, M. A., *Ibid.*, 1976, 16, 147.
5. Biradar, N. S. and Angadi, S. D., *J. Inorg. Nucl. Chem.*, 1976, 38, 1405.
6. Prager, B., Jacobson, P., Schimdt, P. and Stern, D., *Belstein hand buch der Organichen Chemie*, 1931, 14, 323.
7. Sacooni, L. and Ciampolini, M., *J. Chem. Soc.*, 1964, p. 276.
8. Aggarwal, R. C. and Narang, K. K., *Inorg. Chim. Acta*, 1973, 7, 651.

CONTRIBUTION TO THE INDIAN CAPNODIACEAE

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INTRODUCTION

DURING the fungal survey of some of the forests of Western Ghats the authors collected a number of plants infected with sooty moulds. Critical study revealed that they belong to the genus *Capnodium* Mont. Batista and Cifferi[1] (1963) have recognised 13 species of this genus distributed throughout the world, based on the morphology of perithecia, asci and ascospores. From India 12 species are recorded (Butler and Bisby[2], 1931 and 1962), but their identity is doubtful. It is therefore worthwhile to revise the Indian species of *Capnodium*, in the light of Batista and Cifferi's work (1963).

The authors made intensive collection of the species of *Capnodium* and the specimens have been deposited in the mycological herbarium of the Botany Department, Shivaji University. This paper deals with two new species of *Capnodium* Mont, collected during their studies.

(1) *Capnodium batistae* sp. nov.

Mycelium moniliform, septate with subglobose cells 8·5–10 × 4·5–8 μ, usually dark brown, forms subiculum on the leaves.

Pycnidia flasklike, occasionally round, when flasklike often branched, narrow at the upper part and swollen at the middle part, dark brown, 400–520 μ in height, 32–40 μ in breadth at the middle part and 16–18 μ in breadth at the upper part. Pycnidiospores hyaline 1–1·5 μ, round.

Perithecia dark brown, 80–96 × 75–85 μ, oval to round, but slightly narrow at the tip and arise usually from the base of the pycnidia. Perithecial wall made up of polygonal cells 4·7–4·5 × 6–6·5 μ. Asci elliptical, bitunicate and 8-spored, 24–28 × 10–11·5 μ, sessile, aparaphysate. Ascospores elliptical to elongate, with 2 transverse septa but without vertical septa, hyaline, slightly pointed at the tips, 8–9 × 1–1·5 μ.

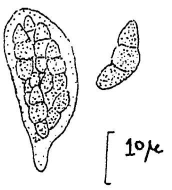

FIG. 1

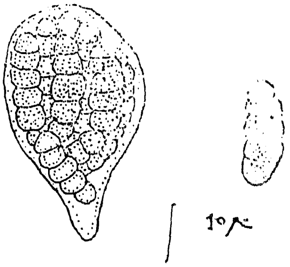

FIG. 2

FIGS. 1–2. Fig. 1. Ascus and ascospore of *Capnodium batistae*. Fig. 2. Ascus and ascospore of *Capnodium kamatii*.

On the leaves of *Ficus carica* L. (Urticaceae) Agri. College Campus, Kolhapur, collected by D. K. Kulkarni on 7–7–1976. Maintained under reference number Cap. No. 1 (Holotype).

Table I clearly shows the difference between new species on *F. carica*, L. and *C. anonae* Pat. which is reported on *F. glomerata*, L., *F. bengalensis*, L., *F. retusa*, L. and *F. bengamina*.

Latin diagnosis.—Mycelium moniliformae septatae cellulis subglobosae, magnit 8·5–10 μ × 4·5–8 μ, plerumque atro-brunneis, subiculum formentibus in foliis.

Pycnidia ampuliformiae, raro sphaerica, ubi ampuliformia seape ramasae, angustatae ad apicem, et inflata, bulbosa, ad median; atro-brunneis 400–520 μ alta, 32–40 μ lata ad median, 16–18 μ lata ad apicem. Pycnidiosporae hyalinae, globosae 1–1·5 μ in diam.

TABLE I

Comparison between C. anonae *Pat. on various* Ficus *hosts and* C. batistae

Cap. sps.	Height of Perithecia	Width of Perithecia	Asci	Ascospores
1. *C. anonae* Pat. on various *Ficus* hosts except *F. carica*, L.	130–380 μ	65–80 μ	50–58 × 24–26 μ	14–22·5 × 7·5–11 μ brown 1–4 vertical septa.
2. *C. batistae* sp. nov. on *F. carica*, L.	80–96 μ	75–85 μ	24–28 × 10–11·5 μ	8–9 × 1–1·5 μ hyaline, 2 transverse septa, no vertical septa.

Perithecia atro-brunneis, 80–96 × 75–85 μ ovoidea vel spherica, leviter angustata ad apicem, formentae plerumque ad basim, pycnidis. Peridis cellulis polygonis, 4·7–4·5 × 6–6·5 μ. Asci bitunicatae, ellipsoideae, octosporae 24–28 × 10–11·5 μ. Sessilae, aparaphysatae. Ascosporae ellipsoidea vel elongatae, bi-septatae, verticalis-septa, nullae, hylinae 8–9 × 1–1·5 μ..

This species is described after Professor A. C. Batista, the well-known Mycologist.

(2) *Capnodium ramatii* sp. nov.

Mycelium amphiphyllous, septate, constricted, cylindrical, branched, not hypopodiate 6–7·4 × 9–10 μ forms subiculum on the leaves and petioles.

Pycnidia globose to subglobose 90–110 μ in diameter, blackish brown. Pycnidiospores hyaline 3–4·5 μ, round.

Perithecia globose to oval 100–112 μ in height. Perithecial wall made up of slightly round cells, 4–6·5 μ, Asci hyaline, elliptical to ovoid bitunicate sessile 8-spored, 40–42 × 14–16·5 μ, Ascospores 4 septate blunt at the tip, 16–19 × 3–3·5 μ.

On the leaves of *Mimusops elangi* L. (Sapotaceae) at Panhala collected by D. K. Kulkarni on 12–5–1976. Maintained under reference number Cap. No. 2 (Holotype).

Latin diagnosis.—Mycelium amphiphyllae, septatae, constricti, cylindracea, ramosis, non hypopodiata, 6–7·4 × 9–10 μ, subiculum formentibus in foliis et petioli.

Pycnidia globosa, vel subglobosa, 90–110 μ, in diam. atrobrunneis, pycnidiosporae sphericae, hyalinae, magnit 3–4·5 μ.

Perithecia globosa, vel ovoidea, 100–112 μ alta, peridis, cellulis rotundatae, 4–6·5 μ, Asci hyalineae, ellipsoidea vel ovoidea, bitunicatae, sessils, octosporae, 40–42 × 14–16·5 μ, Ascosporae quadriseptatae, apicem obtusae, magnit 16–19 × 3–3·5 μ.

The species is described after well-known Indian Plant Pathologist Prof. M. N. Kamat.

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1. Batista, A. C. and Cifferi, R., *Saccardoa*, 1963, 2, 1.
2. Butler, E. J. and Bisby, R., *Fungi of India*, 1931, revision 1962.
3. Saccardo, P. A., *Sylloge Fungorum*, 1882, 1, 33.

INDIAN PHARMACEUTICAL CONGRESS

The Twenty-ninth Session of the Indian Pharmaceutical Congress will be held at Andhra University, Waltair, from 28th to 31st December, 1977. A steering committee with Shri M. R. Appa Row, Vice-Chancellor, Andhra University, as Chairman and Prof. V. Subba Rao, Head of the Department of Pharmaceutical Sciences, Andhra University, as Organising Secretary will work out the details of the Congress. Further information can be had from the Organising Secretary.

LETTERS TO THE EDITOR

ON GOMIDE'S EXPANDING UNIVERSE WITH MATTER INJECTION

1. *Gomide's Model*

Gomide[1] has recently considered matter injection, localized in cosmic radio sources as against Hoyle's[2] creation of matter in empty space, into the observable homogeneous isotropic expanding universe with positive curvature. He accounts for Mach's principle in Whitrow's[3] sense by writing

$$\kappa \mathrm{M}(t)/8\pi \mathrm{R}(t) = (\pi/4)\,\alpha \simeq 1 \tag{1}$$

where κ is Einstein's gravitational constant ($\kappa = 8\pi G/c^2$), $\mathrm{M}(t)$ is the total mass of the closed universe, $\mathrm{R}(t)$ is the radius of curvature of the closed universe and α is a constant which should be greater than three so as to keep $\mathrm{M}(t)$ convergent. Unlike Jordan[4] and Dirac[5] he retains G as constant and instead assumes $\mathrm{M}(t)/\mathrm{R}(t) =$ constant.

He writes, for a homogeneous isotropic and expanding universe, the Robertson-Walker line-element, with positive curvature as

$$ds^2 = c^2dt^2 - \frac{\mathrm{R}^2(t)}{(1+r^2/4)^2}\,(dr^2 + r^2d\theta^2 + r^2\sin^2\theta\, d\phi^2) \tag{2}$$

and his creation field is defined by

$$\left.\begin{array}{l}\lambda_i = \lambda(t)\,(1,\ 0,\ 0,\ 0),\ i = 0,\ 1,\ 2,\ 3)\\ \lambda_0 = \lambda(t) \geq 0\end{array}\right\} \tag{3}$$

$$\lambda_{i;j} = \lambda_{ij} = \frac{\partial\lambda_i}{\partial x_j} - \lambda_k\,\Gamma^k{}_{ij}. \tag{4}$$

The field equations are written as

$$\mathrm{R}_{ij} - (1/2)g_{ij}\,R + g_{ij}\Lambda + \lambda_{ij} = -\kappa T_{ij} \tag{5}$$

where Λ is the cosmological constant. In view of the relation (1) we can write

$$\kappa\rho = \alpha/\mathrm{R}^2 \tag{6}$$

where ρ is the density of the closed universe.

Since injection of matter into the closed observable universe is presumed to have been localized in the 'Source-Holes' of cosmic radio sources, $\mathrm{M}(t)$ and $\mathrm{R}(t)$ (the universe is closed) should be convergent and $\lambda(t)$ should represent a dying out creation process. So we have

$$\left.\begin{array}{l}\mathrm{R}(0) < \mathrm{R}(t) < \mathrm{R}(\infty) = \xi > 0\\ \mathrm{R}'(t) > 0,\ \mathrm{R}'(\infty) = 0\end{array}\right\} \tag{7}$$

and

$$\left.\begin{array}{l}\lambda(0) > \lambda(t) \geq 0,\ \lambda(\infty) = 0\\ \lambda'(t) < 0,\ \lambda'(\infty) = 0.\end{array}\right\} \tag{8}$$

The expressions for density and pressure are as follows:

$$\kappa\rho = \frac{3}{\mathrm{R}^2} + \frac{3\mathrm{R}'^2}{c^2\mathrm{R}^2} - \frac{\lambda'}{c^2} - \Lambda \tag{9}$$

$$\kappa p = \frac{\lambda\mathrm{R}'}{c^2\mathrm{R}} - \frac{2\mathrm{R}''}{c^2\mathrm{R}} - \frac{\mathrm{R}'^2}{c^2\mathrm{R}^2} - \frac{1}{\mathrm{R}^2} + \Lambda. \tag{10}$$

All through, a dash indicates differentiation with respect to t. It could be seen from the inequalities (7) and (8) and the relations (9) and (10) that $\Lambda < 0$ and $\alpha > 3$:

We shall now seek a solution, consistent with (7) and (8), of the following equation

$$\frac{3-\alpha}{\mathrm{R}^2} + \frac{3\mathrm{R}'^2}{c^2\mathrm{R}^2} = \frac{\lambda'}{c^2} + \Lambda. \tag{11}$$

2. *Our Solution*

We shall now give a solution of the equation (11) satisfying the conditions (7) and (8) which ensure a convergent $\mathrm{M}(t)$ and a dying out creation process.

Let us consider

$$\mathrm{R} = \xi\,\frac{t}{\sqrt{t^2+\tau^2}} \tag{12}$$

where τ is a constant having the dimensions of time. Clearly $\mathrm{R}(t)$ satisfies (7). Now (11) would yield

$$\frac{\lambda}{c^2} = \beta - \left(\Lambda + \frac{\alpha-3}{\xi^2}\right)t - \frac{1}{t}\times\left(\frac{3}{c^2} + \frac{(3-\alpha)\,\tau^2}{\xi^2}\right) - \frac{3t}{2c^2\,(t^2+\tau^2)} - \frac{9}{2c^2\,\tau}\tan^{-1}(t/\tau). \tag{13}$$

Hence in view of (8) we determine

$$\beta = \frac{9\pi}{4c^2\tau} \tag{14}$$

$$\frac{3}{c^2\tau^2} = \frac{\alpha-3}{\xi^2} = -\Lambda \tag{15}$$

and so $\lambda(t)$ finally takes the form

$$\lambda = \frac{3}{2}\left(\frac{3\pi}{2\tau} - \frac{t}{t^2+\tau^2} - \frac{3}{\tau}\tan^{-1}(t/\tau)\right). \tag{16}$$

The pressure is throughout negative, $p = -\infty$ at $t = 0$ and $p = \Lambda - 1/\xi^2$ at $t \to \infty$. The negative pressure, as argued by Gomide[1], sustains the pouring of matter into the observable universe. The negative values of Λ and p are characteristic to this model.

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---

1. Gomide, F. M., *Nuovo Cimento*, 1972, **B 12**, 11.
2. Hoyle, F., *Mont. Not. Roy. Astr. Soc.*, 1948, **108**, 372.
3. Whitrow, G. J., *Nature*, 1946, **158**, 165.
4. Jordan, P., *Ibid*,, 1949, **164**, 637.
5. Dirac, P. A. M., *Proc. Roy. Soc.*, 1938, **A 165** 199.

DETERMINATION OF RARE EARTH IMPURITIES IN PRASEODYMIUM OXIDE AND OXALATE BY X-RAY FLUORESCENCE TECHNIQUE

PURE praseodymium contains neighbouring elements lanthanum, cerium, neodymium and samarium in trace levels as impurities. Fassel *et al.*[1] have developed an optical emission spectrographic (OES) method for the determination of first three impurities. Radwan[2] and Murty[3] have extended the OES analysis to all the four elements. In OES technique, even after the use of high resolving power spectrographs, the line overlaps between impurity lines and matrix lines are frequent because of the richness of the spectrum lines. On the other hand, in the X-ray fluorescence (XRF) technique, the analysis is simple since the number of lines in X-ray region is small and line overlaps are few. Using XRF, Lytle and Heady[1] have reported a method for the determination of four rare earth impurities named above. They presented the oxide powder sample to the helium purged spectrometer and using fixed count method obtained a minimum determination limit (MDL) of 0·01%. To obtain this MDL they had to use long counting times of upto 600 seconds.

We have developed an XRF method for the determination of these impurites employing a vacuum path spectrometer and fixed time counting. The sample in our method is taken as an oxalate and presented to the X-ray instrument in the form of a 1¼″ diameter double layer pellet. The double layer form of sample presentation has the advantage that the sample requirement is minimised while retaining the advantages of pelletising, *viz.*, presenting a reproducible surface of uniform density and similar geometry to the primary X-rays. Because of the improved form of sample presentation the required MDL was attained even with a small counting time of 100 seconds.

A Philips ultrastable generator PW 1140 is used to supply power to a Philips 3 kW tungsten target tube, operated at 60 kV and 35 mA, which supplies the primary X-rays to irradiate the sample. The secondary X-rays generated in the sample are dispersed by a LiF (200) crystal in a Philips semi-automatic X-ray spectrometer PW 1220. Fine collimator (160 μm) is used for X-rays and the path is evacuated to a vacuum of 0·5 torr. The intensities of L_α lines of lanthanum, cerium and neodymium and $L_{\beta 1}$ line of samarium are counted for 100 seconds each by a flow proportional counter. The analysis lines used are found to be free from the interference of matrix lines of praseodymium. Under these conditions the impurities can be determined in the range 0·01–1%.

Standards are prepared synthetically by adding impurity rare earth nitrate solutions to praseodymium nitrate solution. Five standards containing 0·01, 0·025, 0·1, 0·5 and 1% of each impurity in praseodymium oxide are prepared. These solution standards are precipitated by a saturated solution of oxalic acid. The precipitate is taken on a filter-paper and dried. 400 mg of dry oxalate is mixed with 400 mg of boric acid binding material. The mixture is ground in an agate mortar and pressed to form a 1¼″ diameter pellet over a boric acid backing pellet.

The sample, if in oxide form, is converted to oxalate through its nitrate solution. The sample is then handled similar to standards.

The precision is calculated by evaluation of percent standard deviation (SD%) using the formula

$$\mathrm{SD\%} = 100\,(\sigma \mathrm{N}_p{}^2 + \sigma \mathrm{N}_b{}^2)^{\frac{1}{2}}/(\mathrm{N}_p - \mathrm{N}_b)$$

where

$$\sigma\,\mathrm{N}_p{}^2 = \Sigma d_p{}^2/n - 1$$

and

$$\sigma \mathrm{N}_b{}^2 = \Sigma d_b{}^2/n - 1.$$

In this formula $\Sigma d_p{}^2$ and $\Sigma d_b{}^2$ represent the sum of the squares of differences of individual peak counts from mean of replicate measurement of peak counts (N_p) and individual background counts from mean of replicate measurement of background counts (N_b) respectively. 'n' represents the total number of values taken for calculations which was 15 in our case. From our calculations it is found that standard deviation is concentration dependent; precision being better at higher concentrations. The SD% values for each element and various concentrations are given in Table I.

The theoretical minimum detection limit (TMDL) defined as the concentration discernible above the background radiation N_b is calculated on three sigma basis using the formula

$$\mathrm{TMDL} = 3\,\sqrt{\mathrm{N}_b} \times \text{concentration/net counts}.$$

The TMDL calculations and their values are given in Table II.

TABLE I

Precision

Sl. No.	Standard %	Per cent		Standard Deviation	
		La	Ce	Nd	Sm
1	1·0	0·98	0·35	0·51	0·16
2	0·5	1·03	0·69	0·74	1·7
3	0·1	3·4	7·7	2·5	7·8
4	0·025	17·1	7·9	9·1	7·7
5	0·01	42·0	37·4	21·1	25·7

TABLE II

Theoretical minimum detection limits (TMDL)

Sl. No.	Element	Background counts N_b	$\sigma = \sqrt{N_b}$	3σ	*Net counts for 100 sec.	TMDL ppm
1	La	9,430	97	291	2,239	32·5
2	Ce	19,419	139	417	3,575	29·2
3	Nd	32,052	179	537	6,148	21·8
4	Sm	25,652	160	480	3,375	35·6

* Net counts are for 250 ppm standard.

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1. Fassel, V. A., Cook, H. D., Krotz, L. C. and Kehres, P. W., *Spectrochim. Acta*, 1952, **5**, 201.
2. Radwan, Z., *Chem. Anal.*, (*Warsaw*), 1971, **16**, 189.
3. Murty, P. S., Bhabha Atomic Research Centre, Trombay, Bombay (unpublished).
4. Lytle, F. W., and Heady, H. H., *Anal. Chem.*, 1959, **31**, 809.

MESOMORPHIC BEHAVIOUR OF SOME NAPHTHALENE SCHIFF BASES: 4-*n*-ALKOXY-1-NAPHTHYLIDENE-*p*-*n*-BUTOXYANILINES

MESOMORPHISM is exhibited mainly by compounds with relatively rigid, polar, rod-shaped molecules that tend to be oriented with their long axes parallel because of the intermolecular attractive forces. It is generally found that nematic compounds have high melting points, because, the molecular characteristics that are necessary for nematic mesomorphism, also produce stable crystalline lattices. For practical applications nematic mesophases which exist near room temperature are desirable. Pure low melting mesomorphic compounds can be prepared by the introduction of dissymmetry in the molecule or by the introduction of lateral substitutions in the molecule.

The introduction of a naphthalene nucleus into a potentially mesomorphic compound should affect its mesomorphic behaviour considerably. Dave *et al.* have observed that the introduction of a 1, 4-substituted naphthalene nucleus considerably reduces the mesomorphic properties of the compounds.[1,2] To further investigate this phenomenon, the following Schiff base compounds of the series 4-*n*-alkoxy-1-naphthylidene-*p*-*n*-butoxyanilines have been synthesized by condensing various 4-*n*-alkoxy-1-naphthaldehydes[1] with *p*-*n*-butoxyaniline[3]. The mesomorphic properties of these compounds have been investigated under a Leitz Ortholux Polarizing Microscope, equipped with Leitz heating stage. The melting points and transition temperatures of these compounds are given in Table I. The combustion analysis for C, H and N for these compounds agree well with their calculated values.

TABLE I

Compound	Alkyl Group	Solid-nematic	Nematic-Isotropic or Solid-Isotropic	
1	CH_3	..	91·0	
2	C_2H_5	(70·5)	88·0[a]	84·0[b]
3	C_2H_7	(56·5)	80·0	
4	C_4H_9	(70·5)	87·5	
5	C_5H_{11}	(61·5)	80·0[a]	74·0[b]

Figures in parenthesis indicate monotropy.
a, stable modification.
b, metastable modification.

The compound No. 1 is non-mesomorphic; the rest are monotropic nematic in nature. Compounds No. 2 and 5 exhibit two solid modifications. The metastable solid modification C_{II} is obtained by a sudden cooling of the isotropic liquid. Both the solid forms on heating give the isotropic liquid. The metastable solid modification melts at a lower temperature than the stable solid modification C_I. In both the cases the isotropic liquid cools to give a monotropic nematic phase. These can be represented as follows in Fig. 1.

The non-mesomorphism of compound No. 1 can be attributed to its relatively high melting point. It is observed that the melting points and transition temperatures of these compounds are higher than the corresponding members of the series 4-*n*-alkoxy-1-

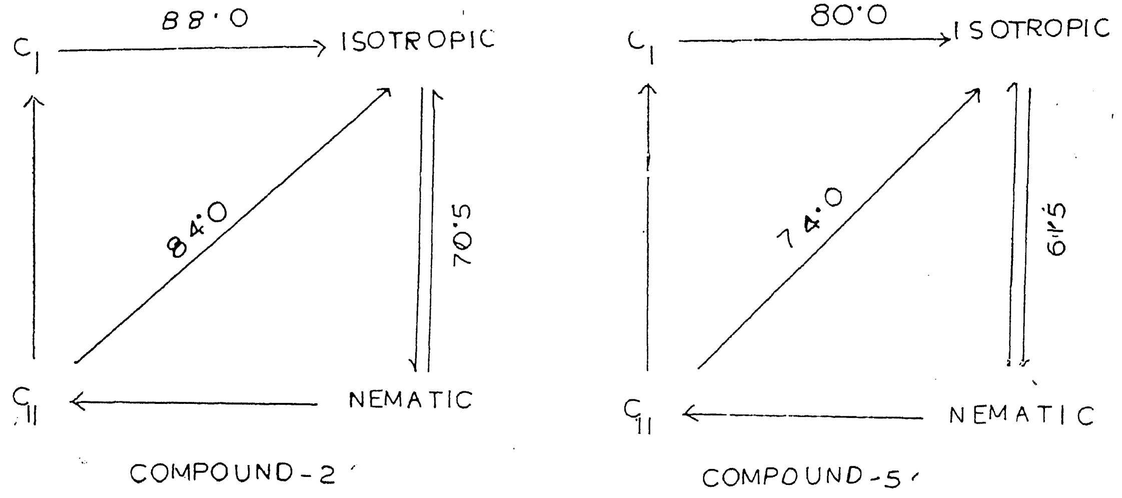

FIG. 1

naphthylidene-*p*-*n*-amyloxyanilines. This compares with the results of Dave and Patel in their study of the corresponding benzylidene series[4]. Further work is in progress.

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1. Dave, J. S., Kurian, G., Prajapati, A. P. and Vora, R. A., *Mol. Cryst. Liquid Cryst.*, 1971, **14**, 307; *Indian J. Chem.*, 1972, **10**, 754.
2. —, and Prajapati, A. P., *Pramāṇa, Suppl. No.* 1, 1975, p. 435; *Curr. Sci.*, 1976, **45**, 95.
3. Gutekunst, G. O. and Gray, H. L., *J. Amer. Chem. Soc.*, 1922, **44**, 1741.
4. Dave, J. S. and Patel, P. R., *Mol. Cryst.*, 1966, **2**, 103.

EFFECT OF SOLVENT ON ABSORPTION AND FLUORESCENCE SPECTRA OF ISOMERIC TRIFLUOROMETHYL BENZONITRILE ($C_6H_4CF_3CN$)—ESTIMATION OF DIPOLE MOMENT CHANGE

THE study of solvent effect upon absorption and fluorescence spectra is one of the methods to understand nature of the electronic excited and ground states. In solutions, the position of the fluorescence bands under certain circumstances show marked solvent effect. It is usually the solvent polarity which principally affects the π-π transitions and particularly if such transitions have some mixed intramolecular charge transfer contribution. If the charge transfer character is great, hydrogen bonding solvent as well as the polar solvent shift fluorescence to longer wavelength[1].

Table I giving the absorption and fluorescence wavelength maxima of isomeric trifluorobenzonitriles ($C_6H_4CF_3CN$) in ethanol, water and cyclohexane shows the effect of solvent on the corresponding spectra. In these molecules the wavelength of fluorescence maxima shows a considerable shift in going from non-polar to polar solvent. It is clear from these results that these molecules become more polar in the excited state than in the ground state showing larger dependency of fluorescence band maxima on solvent than absorption.

TABLE I

Solvent	Wavelength ($m\mu$)					
	Absorption			Fluorescence		
	Ortho	Meta	Para	Ortho	Meta	Para
Cyclohexane	271·0	269·9	270·7	327	329	332
Water	271·4	270·2	272·5	339	342	343
Ethanol	271·5	270·5	272·0	340	342	344

Further, the study of the wavelength of absorption and fluorescence spectra in different solvents can be utilized for an estimation of the order of magnitude of the dipole moment change from the ground state to the first excited singlet state. The relation for such an estimation is given by (using Oshika's theory)[2, 3]

$$-hc\,(\nu_f^m - \nu_a^m) \simeq \text{constant} + 2\,(D-1)/2\,D+1 \\ - 2\,(n^2-1)/2n^2+1 \times (\mu_e - \mu_g)^2/a^3$$

where ν_f^m and ν_a^m are the fluorescence and absorption frequencies (in wave numbers) of corresponding maxima corresponding to the *m*-th state, in case the (0–0) transitions are not observable (which is generally the case in solution study). D is the dielectric constant and *n* the refractive index of the solvent, μ_e and μ_g are the dipole moments of the solute in the electronic excited and ground singlet states respectively. The quantity '*a*' is the radius of the solute cavity in the solvent dielectric (as given by Onsagar).

The estimated quantity $(\mu_e - \mu_g)^2/a^3$ in isomeric trifluoromethylbenzonitriles is found to vary in the order para $<$ meta $\approx$ ortho. Under an assumed value of '*a*' about 2A, the estimated values of $(\mu_e - \mu_g)$ are 1·47, 1·69 and 1·68 debye units in para, meta and ortho isomers respectively. Corresponding calculations in case of trifluorotoludines give $(\mu_e - \mu_g)$ as 2·3 and 2·5 debye units in meta and para isomers respectively. It is significant to observe that in case of trifluorotoludines, the change in dipole moment is larger than that in case of trifluoromethylbenzonitriles. In the first case, the substituents are electron withdrawing *vs.* electron donating, whereas in the latter both substituents are electron withdrawing. Secondly in the toludines, the charge transfer states make a contribution to the excited state. Both these factors contribute to the greater dipole moment change in case of amines than in nitriles.

The results obtained by this method show the change in the dipole moment which in turn contribute substantially to the understanding of the nature of the states.

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1. Schulman, S. G., *Fluorescence News*, 1972, 6 (4), 1.
2. Becker, R. S., *Theory and Interpretation of Fluorescence and Phosphorescence*, Wiley-Interscience, New York, 1969.
3. Lippert, E., Z. *Physik*,, *Chem.* (N.F.), 1956, 6, 125.

FIRST RECORD OF GOLDEN OOLITES FROM THE BEDESAR FORMATION, JAISALMER BASIN, RAJASTHAN

IN the Jaisalmer basin, Rajasthan, the sequence of rock formations is as summarised in Table I.[2,1,7,8]

TABLE I

Lower to Middle Eocene	Bandha Formation	75 m
Paleocene to Lower Eocene	Khuiala Formation	100 m
Paleocene	Sanu Formation	75 m
	—Unconformity—	
Lower to Middle Cretaceous	Abur Formation	60 m
Lower Cretaceous	Parihar Formation	305 m
	—Unconformity—	
Jurassic	Bedesar Formation Baisakhi Formation Jaisalmer Formation Lathi Formation	61 m 150 m 300 m 360 m
Triassic	Non-calcareous Sandstone and Claystone (Sub-surface)	260 m

The rock formations are correlatable to those in Kutch; Lathi to Patcham, Jaisalmer to Chari, Baisakhi to Katrol, Bedesar to Upper Katrol-Lower Umia, Parihar to Umia, and Abur to Ukra[6]. "Golden Oolite" occurs in the Chari as a few layers "constituting the DIADEMATUS ZONE (*Indo-cephalites diadematus*), which is a calcareous oolite, the grains of which are coated with thin films of ferric oxide giving them a golden colour"[2]. It is quite thick, locally disturbed and often wanting[5]. The Variegated Series of Salt Range and its Trans-Indus continuation also contain thin layers of "Golden Oolite", similar to the Kutch rocks, associated with argillaceous limestone, and rust-coloured sandstones, and formed as a result of the ferruginisation of an oolitic limestone[5]. The Jaisalmer Formation has oolites in its upper part noted at several localities in the Jaisalmer basin[3]. Near Hamira (27° 0″ : 71° 4′ 30″), golden calcarenite of 20 cm thickness has also been recorded, which shows coarse grained limestone with metallic lustre, golden yellow coloured coatings on tiny gastropods upto 2 mm size; these are restricted to the lower part of the Jaisalmer Formation. The "Kuldhar Member" named as the topmost portion of the Jaisalmer Formation has oolite bands[1]. "Golden Oolites" are also found at the same horizon in the Jurassic of Europe. Thus the oolitic zone serves as a marker horizon in the Middle Jurassic strata of Western India and Pakistan.

This note records, for the first time, oolites from the Bedesar Formation. The localities of conspicuous occurrence are the hill ·807 due south of Kumbhar Kotha (26° 44′ 25″: 70′ 44′ 55″), Chinchalci Dungar (26° 40′ 45″: 70° 34′ 40″), east of Malan-ka-Than (Dhanneli) (26° 35′ 50″: 70° 34′ 30″), 4·5 km ESE of Bida 26° 42′ 30″: 70° 31′ 35″), and 0·5 km north-west of hill ·731 in the district Jaisalmer. Systematic geological mapping has revealed that these oolites form a persistent horizon and can be traced all over the area in the lower portion of the Bedesar Formation.

South of Kumbhar Kotha, on western side of the hill ·807, bands of yellow and golden yellow oolites, oolitic ironstone, and oolitic marlstone occur in 9 m thick sequence of ferruginous sandstone and ironstone. They are confined mainly to the upper 5 m section. Greyish oolites also occur as a distinct band in the brownish weathered calcareous marlstone in the lower 4 m sequence. This horizon is located in the lowermost portion of the Bedesar Formation. Shallow digging in the area has revealed the occurrence of gypseous bentonitic clay, which is indicative of the occurrence of Baisakhi Formation.

A well-exposed 75 cm thick bed is recorded at the base of 4 m thick sequence, comprising ironstone, calcareous ironstone, ferruginous sandstone, and sandy ironstone, along with bands of hematite and ochreous sandstone, exposed on the eastern side of Chinchalei Dungar.

The oolites form up to 35% of the quartzose rock and occur in a ferruginous cement (Figs. 1, 2, Plane Polarized Light, ×32). They are confined to the ferruginous, vitreous, red, brown, and maroon coloured grits and gritty sandstone, interstratified with thin layers of hematite, and hematitic matter. The yellow colour is due to ferric oxide coating on calcareous oolites, showing one concentric layer (Fig. 1) or more (Fig. 2) embedded in a reddish or maroon coloured ferruginous matrix. The nuclei of oolitisation is quartz and calcite. The rock is likely to be completely overlooked in the field, as has been done by previous workers, unless examined very closely, and traced laterally. The rock formation forms cappings of the Baisakhi Formation, separated as isolated scanty hillocks in the vast desertic terrain. The oolites owe formation evidently due to the intense ferruginisation of the beds.

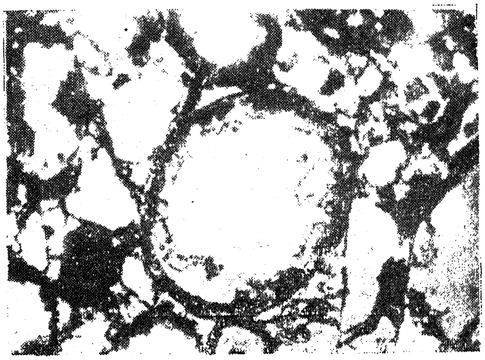

FIG. 1

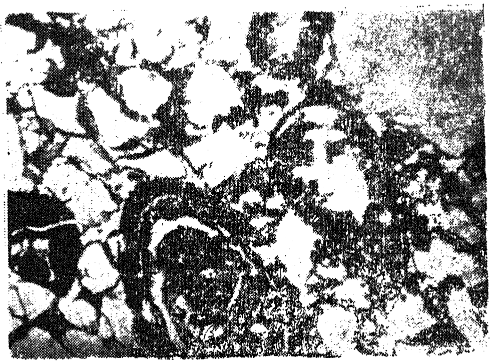

FIG. 2

Western Rajasthan forms part of the shelf in which Mesozoic and Tertiary sedimentation took place, the basin extending from Kutch in the south to the Salt Range, Pakistan, in the north. Oolites have not been reported so far from the beds correlatable to the Bedesar Formation, *i.e.*, Upper Katrol–Lower Umia beds of Kutch, or of equivalent beds in Pakistan. This find, therefore, is of significance in indicating the configurational set-up of the Jaisalmer basin.

The association of oolite horizons with the interbedded sequence of grits, sandstones, and ironstones is indicative of the fact that the shore line was oscillating between near-shore to neritic environments. The oolites were formed in the near-shore environment where high energy conditions were prevalent. Their prevalence also exhibits unstable tectonic condition in the basin.

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---

1. Dasgupta, S. K., *Indian Jour. Earth Sci.*, 1975, **2**, 77.
2. Krishnan, M. S., *Geology of India and Burma*, Higginbothams (P) Ltd., Madras, 1968, pp. 361, 367.
3. Madhava Rao, M. R. and Laul, V. P., "Geological mapping in parts of Barmer and Jaisalmer districts," *Unpub. Rep. Geol. Surv. Ind.*, 1975.
4. Pareek, H. S., "Geological Configuration of North-Western Rajasthan," *Workshop on 'The Problems of the Deserts in India'* Sept. 16, Jaipur, 1975, p. 77.
5. Pascoe, Edwin, H., *A Manual of the Gealogy of India and Burma*, Vol. II, Third Edition, Govt. of India Press, Caloutta, 1968, pp. 1134, 1158,
6. Poddar, M. C., *Rep. 22nd Sess. Int. Geol. Cong.*, New Delhi, Pt. I, 1964, p. 126.
7. Swaminath, J. *et al.*, "Progress report of the Geological Survey of the Jaisalmer District and adjacent areas of Rajasthan," *Unpub. Rep. Geol. Surv. Ind.*, Pt. I. 1956–57.
8. Tikku, C. L. *et al.*, *Curr. Sci.*, 1976, **45** (15), 554.

PYRETHRIN SYNERGISTIC ACTIVITY BY THE ESSENTIAL OILS OF A FEW *BLUMEA* SPECIES

Introduction

PYRETHRUM, a nontoxic insecticide, has in recent years developed a lot of interest in controlling household pests particularly after getting synergised in one way or the other. The high degree of synergism was obtained by piperonyl butoxide when used against rice weevils[1–3]. The piperonyl butoxide being a costly imported item, a few substitutes have also been reported for such an activity[4 5].

Thus to assess the synergistic property of different compounds in place of piperonyl butoxide, the present study was undertaken for the essential oils of *Blumea lacera* DC. and *B. malcomii* Hook, plants of family Compositae; against the house flies (one of the standard test insects)[6]. The synergistic properties with pyrethrum have been described and discussed for both the oils.

Experimental

The essential oils extracted from *B. lacera* and *B. malcomii*; Pyrethrum extract containing 20% w/w pyrethrums and technical grade piperonyl butoxide (Fluka, Switzerland) were used throughout the experiments. The different concentrations of pyrethrum taken were ·00125; ·0025, ·005 and ·01% w/v respectively. The mixture of pyrethrums with essential oils and piperonyl butoxide were mixed in the ratio of 1 : 10.

Adult house flies (*Musca domestica* L.) between 3-4 days old reared at 25° C and 75% RH were taken for the various testings. The various testings were done as per British standard methods[6].

One μl solution in acetone of the above-mentioned concentrations was given to the individual narcotised (chilled at 4° C) female fly by topical application with the help of a microsyringe applicator (designed by us in Regional Research Laboratory, Jammu). The mortality was recorded after 24 hours and plotted on log graph. The knockdown of the female flies was determined by peet grady chamber method[7,8].

Results and Discussion

The knockdown and mortality recorded for pyrethrum, *B. lacera* and *B. malcomii* oils and piperonyl butoxide showed a definite variability with reference to various concentrations (Table I). While 0·01% concentration when applied topically showed a mortality of 36·6, 80·0, 60·0 and 100% respectively, the lowest dose of 0·00125% exhibited a percentage mortality of zero, 10·8, 6·6 and 40 respectively (Table II, Fig. 1).

TABLE I

*Incidence of the knockdown percentage in space sprays (Peet grady chamber)**

Concentration %	Pyrethrum		*B. lacera*		*B. malcomii*		Pyrethrum + *B. lacera*		Pyrethrum + *B. malcomii*		Pyrethrum + Piperonyl butoxide	
	Knock-down	Mortality	Knock-down	Mortality	Knock-down	Mortality	Knock-down	Mortality	Knock-down	Mortality	Knock-down	Mortality
0·1	95	100	Nil	Nil	Nil	Nil	100	100	100	100	100	100
0·05	62	60	Nil	Nil	Nil	Nil	100	100	80	100	100	100
0·025	43	47	Nil	Nil	Nil	Nil	83	90	56	52	92	100
0·0125	7	7	Nil	Nil	Nil	Nil	43	43	19	22	60	55

* Average of 5 replicates of 100 female flies/batch. Dose = 55 ml/1000 Cubic Feet.
Knockdown = After 10 min. Mortality = After 24 hours.

TABLE II

Percentage mortality in female house flies against different concentrations by topical application

Concentration %	Pyrethrum	*B. lacera*	*B. malcomii*	Pyrethrum + *B. lacera*	Pyrethrum + *B. malcomii*	Pyrethrum + Piperonyl butoxide
0·01	36·6	Nil	Nil	80·0	60·0	100·0
0·005	20·0	Nil	Nil	63·3	30·0	86·0
0·0025	[illegible]·6	Nil	Nil	50·0	16·6	76·6
0·00125	0	Nil	Nil	10·8	6·6	40·0

The LD50 value for pyrethrum was found to be 0·0115 μg. While LD50 value for piperonyl butoxide + pyrethrum was quite low (0·0017 μg) the highest value was recorded for *B. malcomii* oil + pyrethrum (0·0060 μg; Table III). These values, of course, suggest that synergised pyrethrum by piperonyl butoxide is more active than other synergising items. But then *B. lacera* oil + pyrethrum mixture can easily be compared to the former because of the close LD50 value with the candidate oil. This gets a further support from the synergistic coefficients calculated for all the categories (Table III). While piperonyl butoxide shows a degree of synergism equal to ten (10); *B. lacera* oil mixture revealed a value of seven (7); thereby showing that it is only 30% less active than piperonyl butoxide. However, the mixture of *B. malcomii* oil is exactly 50% less active than piperonyl butoxide. Moreover, the present synergising oils appear more active than the *kerangin*, whose potency has been reported to be quite inferior to piperonyl butoxide[3]. The activity, however, is difficult to compare with Dillapiol, because its activity has not been compared with that of piperonyl butoxide[4].

Table III

Incidence of LD50 values and degree of synergism in B. lacera *and* B. malcomii *oils*
(*Pyrethrum*: *Synergist*; 1:10)

	LD50 (μg/fly)	Degree of Synergism*
Pyrethrum	0·0115	..
Pyrethrum + *B. lacera*	0·0033	7
Pyrethrum + *B. malcomii*	0·0060	5
Pyrethrum + Piperonyl butoxide	0·0017	10

* Ratio of the equitoxic dose of an insecticide alone to an equivalent dose in the presence of synergists (at LD50 values in the present case) experessed as a whole number.

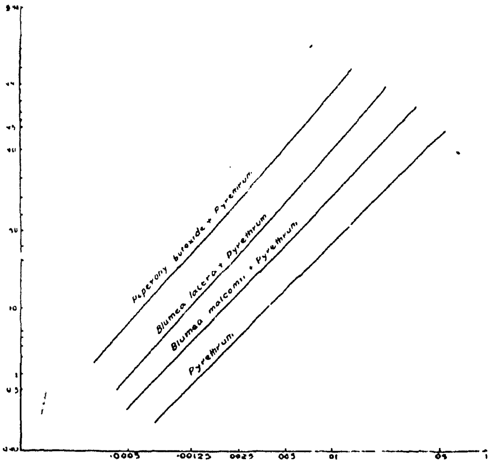

Fig. 1. Log graph showing mortality curves.

Thus on the face of the high degree of synergism by piperonyl butoxide, the activity of the present oils (particularly *B. lacera* oil; degree of synergism = 7) cannot be summarily discarded. The studies on the active principles of both the oils and their chemical nature can further enlighten their synergistic constituents for pyrethrins.

Authors are highly thankful to Dr. C. K. Atal, Director of the Laboratory, for his keen interest during the study. The thanks are also due to Shri B. L. Kaul for technical assistance.

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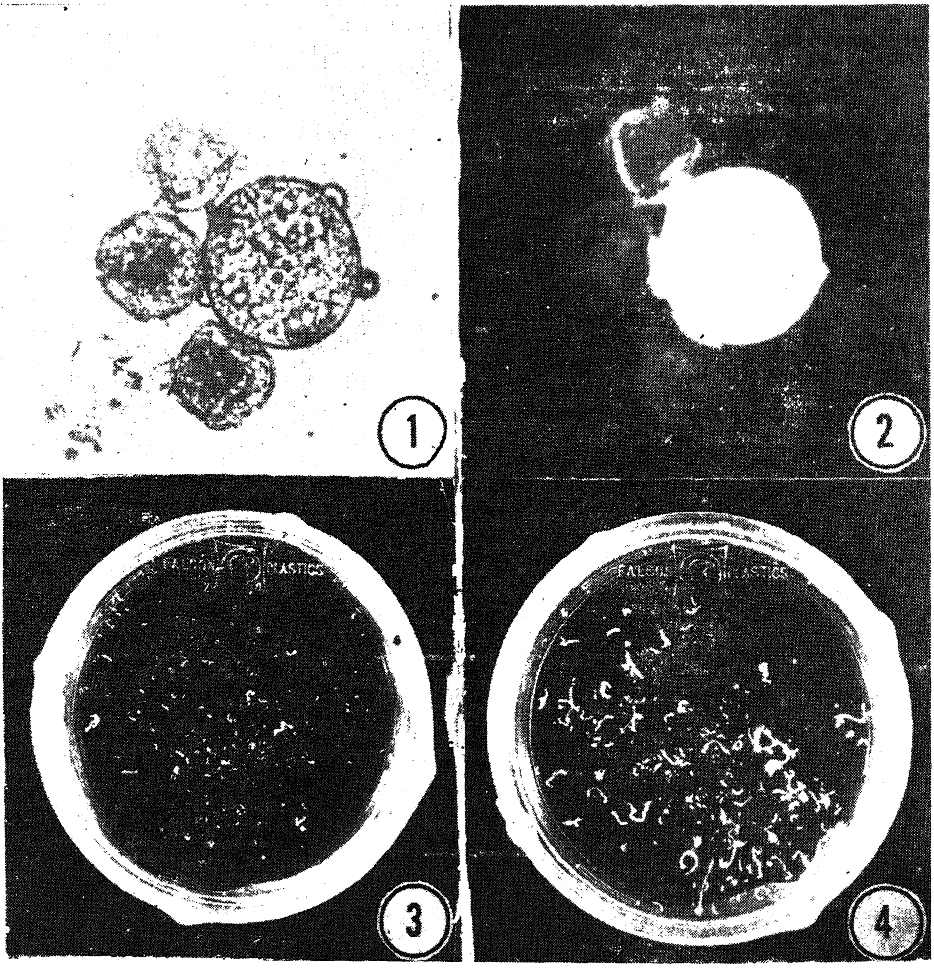
1. Watts, C. N. and Berlin, F. C., *J. Eco. Ent.*, 1950, **43** (3), 371.
2. Goodwin-Bailey, K. F. and Holborn, J. M., *Pyr Post.*, 1952, **2** (4), 7.
3. Schulze, T. L. and Hansens, E. J., *J. Eco Ent.*, 1975, **68** (6), 807.
4. Parmar, B. S. and Gulati, K. D., *Indian J. Ent.*, 1969, **31** (3), 239.
5. Handa, S. K. and Dewan, R. R., *Pyr. Post.*, 1975, **13** (2), 45.
6. Head, S. W. and Winney, R., *Ibid.*, 1972, **11**, 152.
7. Nelson, R. H., In *Method of Testing Chemicals on Insects*, Burgess Pub. Co., USA, 1960, **2**, 34.
8. Busvine, J. R., *A Critical Review of the Techniques for Testing Insecticides*, Commonwealth Agric. Bureaux, England, 1971.

SURVIVAL OF *ATROPA* AND *NICOTIANA* POLLEN-EMBRYOS FROZEN AT − 196° C

The cryobiology of plant cell cultures has recently attracted much attention for a variety of reasons[1,2], especially for long-term storage of tissue cultures. The callus cultures on repeated subculturing for an extended period of time have a tendency to undergo chromosomal variations and changes in the ploidy level[3,4,5]. In this context haploid cultures are particularly known to be highly unstable and within a few weeks they revert to a diploid state. At present no standard method for their preservation is available. In view of the great importance of haploid cells in genetics and mutation studies, their maintenance as haploids is highly desirable. The present communication outlines a cryogenic method for the freeze preservation of haploids, Pollen-embryos obtained from 3 to 5 week-old anther cultures of *Atropa belladonna*[6] and *Nicotiana tabacum*[7] were suspended in their respective culture media containing 5–7% dimethyl sulfoxide as a cryoprotectant. One ml aliquots containing pollen-embryos at various stages of androgenesis were dispensed into sterile plastic ampuoles and cooled at the rate of 1–3° C/min in a liquid nitrogen cylinder (Union Carbide Freezer LR 33 attached with BF-6). After freezing, the ampuoles were thawed at 37° C in a water-bath and the contents washed repeatedly. The viability of the embryos was then determined by their capacity to show fluorescence with fluorescein diacetate[8] (Figs. 1, 2) and also by their growth response in a ynthetic culture medium[9] (Figs. 3, 4).

Globular embryos showed higher survival (31%) as compared to early heart-shaped embryos (9%) and late heart-shaped embryos (2%). This low survival of maturing embryos, like those of old cell suspensions[1], is attributed to their expanded and differentiated cells in contrast to highly cytoplasmic, small sized, non-vacuolated and thin walled cells of the globular embryos. In cultures (Figs. 3, 4) globular and early heart-shaped embryos underwent normal androgenesis and formed haploid plants, while in older embryos only a few cells revived and proliferated to form callus.

These observations on the survival of pollen-embryos at a super-low temperature suggest the possibility of employing cryogenic methods for the establishment



Figs. 1–4. Fig. 1. Suspension of pollen embryos of *Nicotiana tabacum* frozen at −196° C and photographed in tungsten light. Fig. 2. Same photographed under ultra-violet light after staining with fluorescein diacetate; note the viable globular pollen- embryo showing fluorescence. Fig. 3. Cold-treated pullen-embryos of *Atropa belladonna* at the time of culture. Fis. 4. Same, four weeks after culture; note the growth of embryos and the formation of plantlets.

of gene-banks for conservation of haploids and other germplasm of important plant species.

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1. Bajaj, Y. P. S., *Physiol. Plant.*, 1976, **37**, 263.
2. — and Reinert, J., *Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture* (Eds. J. Reinert and Y. P. S. Bajaj). Springer-Verlag, Berlin-Heidelberg-New York, 1976, p. 757.
3. Sacristàn, M. D., *Chromosoma*, 1971, **33**, 273.
4. Bayliss, M. W., *Nature*, 1973, **246**, 529.
5. D'Amato, F., *Crop Genetic Resources for Today and Tomorrow* (Eds. O. Frankel and J. C. Hawkes), Cambridge University Press, 1975, p. 333.
6. Zenkteler, M., *Experientia*, 1971, **27**, 1087.
7. Nitsch, J. P. and Nitsch, C., *Science*, 1969, **163**, 85.
8. Widholm, J. M., *Stain Technol.*, 1972, **47**, 189.
9. Nitsch, C., *C.R. Acad. Sci. Ser. D*, 1974, **278**, 1031.

INTERSEXUALITY IN THE FRESH WATER TELEOST, *HETEROPNEUSTES FOSSILIS*

ALTHOUGH the teleost fishes have been recognised as unisexual yet spontaneous intersexuality or hermaphroditism is known to occur in a few teleosts (Atz[1], 1964). The degree of intermingling of the ovarian and testicular tissues varies in different fishes. James[3] (1946) observed, one half of the ovotestis as testicular and the other half as ovarian in *Huro salmoides*. D'Ancona[2] (1950) noticed the gonadial inversion in the gold fish *Sparus auratus*. Sathyanesan[5] (1957) reported the presence of a single oocyte in the intertubular space in the adult testis of *Barbus stigma* while Krishnamurthy *et al.*[4] (1970) observed the immatured oocytes scattered within the testicular tissues of *Rasbora daniconius* and also grouped together in well-defined areas.

A careful study of the serial sections of the gonad of *Heteropneustes fossilis* by the authors revealed that the gonad in this fish is an ovotestis. The presence of ovotestis in this fish is very uncommon. The ovotestis (Fig. 1) is found to contain, isolated, one or two immature and mature oocytes lying attached to one side of the adult testis. The immature oocytes are characterised by a clear homogeneous cytoplasm and a big nucleus with numerous small nucleoli. The matured oocytes show the presence of well-developed yolk globules of different sizes with a shapeless nucleus. The testis showed normal features.

The present observations point out a few similarities and dissimilarities with the findings of other workers. The occurrence of oocytes only on one side of the adult testis is in contrast with the findings of Sathyanesan[5] (1957) and Krishnamurthy[4] (1970). Intermingling of the oocytes within the testicular tissues has not been noticed in the ovotestis of *H. fossilis*. The presence of both immature and matured oocytes are again in contrast with the explanations of Krishnamurthy *et al.*[4] (1970) who have observed only immature oocytes scattered within the testicular tissues of *Rasbora daniconius*.

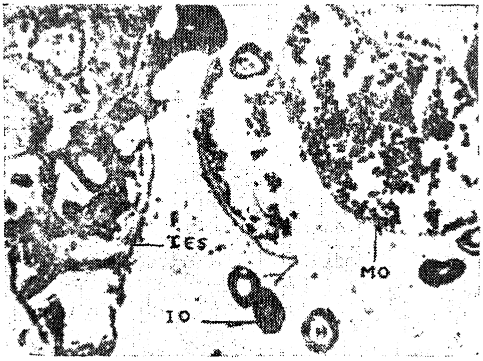

FIG. 1. Section of the Ovotestis of *Heteropneustes fossilis*. Zenker/Iron haemotoxylin, × 300.

(TES, Testis; IO, Immature oocyte; OM, Mature oocyte.

The occurrence of ovotestis in *H. fossilis* cannot be stated at this stage as the phenomenon of hermaphroditism or sex reversal as this fact needs further investigation and elaboration.

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1. Atz, J. W., "Intersexuality in fishes," In: *Intersexuality in Vertebrates Including Man*, Eds. C. N. Armstrong and A. J. Marshal, Academic Press, New York, 1964, p. 145.
2. D'Ancona, *u. CNRS collogue, International No.* 31, *La Differentiation sexuelle chez les vertebres*, 1950.
3. James, F. M., *J. Morph.*, 1946, **79**, 93.
4. Krishnamurthy, C. G., Rajasekarasetty, M. R. and Gopapa Dutt, N. H., *Indian Biologist*, 1970, **2** (1), 80.
5. Sathyanesan, A. G., *Sci. and Cult.*, 1957, **23**, 203.

PELTOTRACHELUS COGNATUS MSHL. (CURCULIONIDAE: COLEOPTERA) A NEW PEST OF ORCHARD CROPS

THE genus *Peltotrachelus* is represented by three species in India and the adults are known to be the defoliators of tree hosts. *Peltotrachelus juvencus* Fst., *P. pubes* Fst., and *P. albus* Poscoe have been reported infesting *Acacia catechu* W. and A., *Citrus aurantium* L., *Tectona grandis* L.f., and *Zizipus jujuba* Lam. in India[1–3] and *P. isabellinus* Boh. infesting cacao in Java[4].

TABLE I

Incidence of Peltotrachelus cognatus *on* M. indica *grafts at Main Research Station, Hebbal, Karnataka*

Month	Old growth (Bottom whorl)		New growth (Top whorl)		No. of weevils observed	
	No. of leaves*	No. of leaves* damaged	No. of leaves*	No. of leaves* damaged	*P. cognatus*	*M. undecim pustulatus* var. *maculosis*
September						
Total	128	14	320	150	26	6
Average	10·66	1·16	26·66	12·50	2·16	0·50
Per cent damage		10·70		46·70		
October						
Total	98	22	306	180	24	4
Average	8·16	1·80	25·5	15·00	2	0·33
Per cent damage		22·44		58·80		

* Observation on 12 plants.

The adults were observed of *P. cognatus* infesting leaves of orchard crops namely *Achras sapota* L., *Anacardium occidentale* L., *Mangifera indica* L., *Prunus avium* L., *P. domesticus* L., *P. persica* Benth. and Hook., *Psidium guajava* L., *Pyrus communis* L., *Pyrus malus* L., *Punica granatum* L., and other tree hosts namely *Diospyros montana* Roxb., *Holoptelia integrifolia* Planch, *Dalbergia lanceolaria* L.f., and *Morus alba* L. for the first time in India. The weevils were found to feed from leaf margin proceeding inward leaving the thick veins and midrib in an irregular fashion (Fig. 1). The adults appeared in large numbers on the above hosts in May and remained on them till November. They were observed congregating on the underside of the leaves during the sunny hours and scattered themselves during cool hours.

During September and October 1976, *M. indica* grafts were found heavily damaged by *P. cognatus* at Main Research Station, Hebbal. A perusal of Table I indicates that the damage was more on the leaves of new growth (46·7 and 58·8%) than on old growth (10·7 and 22·4%). Weevils preferred brittle leaves to very tender ones of new growth. *Peltotrachelus cognatus* and *Myllocerus undecimpustulatus* var. *maculosus* Desb., which occurred together, were both responsible for the damage. However, the numbers of former being four times more than the latter, it can be inferred that *P. cognatus* was responsible for the major portion of the damage (Table I). Observations on *M. indica* grooves around Bangalore also revealed the severity of the damage to the grown-up trees.

The field crops in the vicinity of orchard crops were seldom infested by the species, thus revealing the preference of adults of *P. cognatus* to the orchard crops.

The adults of *P. cognatus* are often mistaken for *M. undecimpustulatus* var. *maculosus* as they look alike. However, the former can be distinguished by the pale colour with three brown coloured transverse wavy lines on the elytra (Fig. 1).

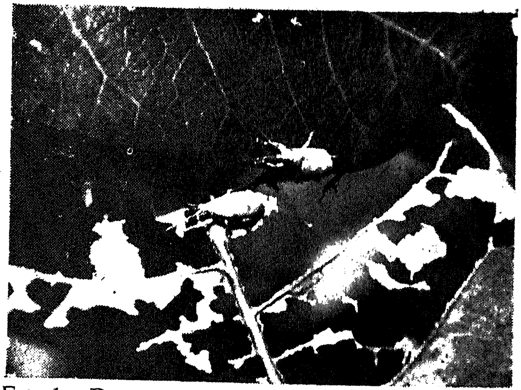

FIG. 1. Damage to the *M. indica* leaves caused by *P. cognatus*.

The authors are thankful to the authorities of British Museum, London, for identifying the weevil.

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1. Bhasin, G. D. and Roonwall, M. L., *Ind. For. Bull.*, 1954, **171** (1), 93.
2. —, Roonwal, M. D. and Balwant Singh. *Ibid.*, 1958, **171** (2), 126.
3. Mathur, R. N. and Balwant Singh, *Ibid.*, 1961, **171** (9), 116.
4. Paerels, J. J., "Overzicht over de voornaanste ziekten,' en plagen van den Cacaboom op Java Tijdschr," *Tijdschr Plantenziekten*, 1924, **30** (3), 51.

MODIFICATION IN THE LABIAL GLANDS OF LARVA OF *SYNTOMIS FORTUNEI* ORZA (SYNTOMIDAE: LEPIDOPTERA)

OUR knowledge of the biology of *Syntomis fortunei* on soybean is due to Singh and Gangrade[3] (1974) and as no significant information is available on the labial glands, an attempt is made here briefly to indicate the modification of these glands in the larvae. The labial glands of the larvae of *S. fortunei* (Fig. 1) are the most characteristic paired appendages associated with the digestive canal, lying partly on its lateral sides in the thoracic segment and partly underneath it from first to third abdominal segments where they usually end. Anteriorly, the two glands enter into the head a little anterior of the prothoracic ganglion, to form a common duct (B) which extends below the hypopharynx to open on the spinneret (A) of the labium. The fillipi's glands are completely absent in this species. In other lepidopterans, *Spodoptera litura* (Shrivastava and Mathur[2], 1963) and *Acherontia styx* (Mukherjee[1], 1962), the fillipi's glands are present in different shapes. Each duct of the glands beyond the head region is narrow, elongate and cylindrical (C) in the prothorax and anterior mesothorax and then assumes a lobular structure (D) through the mesothorax. The lobes are packed closely in the opposite walls of the duct in a slightly slanting position so that the duct appears very closely zig-zag or spiral (Fig. 2, D). Further down the duct is enlarged in diameter to become saccular and smooth walled (E). Such a structure of the labial glands of the larvae of *S. fortunei* is at complete variance with the anterior tubular and posterior S-shaped glands of the larva of *Spodoptera litura* (Shrivastava and Mathur[2], 1963) and greatly convoluted glands of the larva of *Acherontia styx* (Mukherjee[1], 1962).

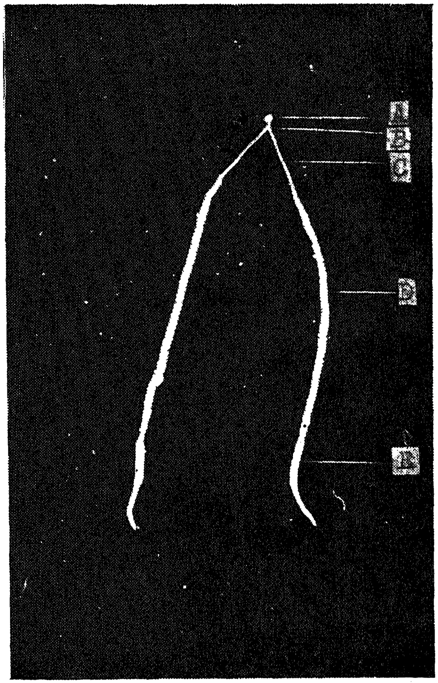

FIG. 1. Labial glands of *Syntomis jortunei* Orza.

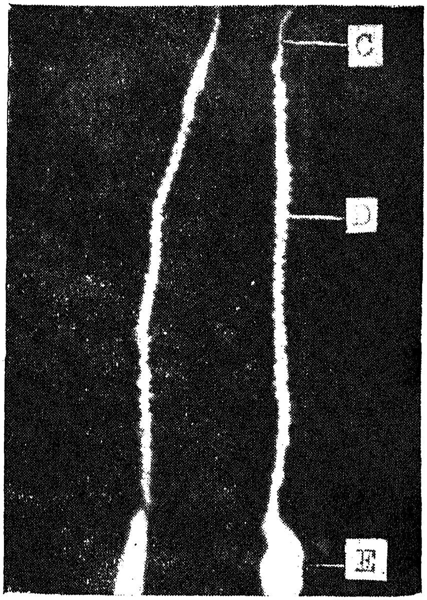

FIG. 2. An enlargement of labial glands of *S. jortunei* showing C, D and E parts only.

This work was done under a financial grant No. FG-IN-412 from the United States, Department of Agriculture, Washington, D.C.

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1. Mukherjee, G. P., *Indian J. Ent.*, 1962, **24**, 94.
2. Shrivastava, B. K. and Mathur, L. M. L., *Ibid.*, 1963, **25**, 116.
3. Singh, O. P. and Gangrade, G. A., *Sci. and Cult.*, 1975, **41**, 269.

NIPTERA INDICA SP. NOV., A NEW GENERIC RECORD TO INDIA

A RARE discomycete was collected on three different hosts from three separate localities of Maharashtra, thereby showing wide distribution of this fungus. On examination and critical study, the fungus proved to be an undescribed species of the apothecial genus—*Niptera* Fr.[1] (Dermateaceae). This genus had remained unrepresented in the fungal flora of India so far[5].

TABLE I

Comparison between species of niptera

Species	Apothecia	Asci	Ascospores	Reference
N. lacustris (Fr.) Summa. (type sp.)	..	..	22–30 × 6–9 µm	Saccardo[7]
N. taxi Rea	0·2–0·6 mm	50–60 × 8–10 × µm	8–12 × 4 µm	C. Rea[6]
N. subilata Seaver	1 mm	60–75 × 6 µm	8–10 × 2–2·5 µm	J. Seaver[8]
N. Muelleri-Argovensis Rehm	0·5 mm	30–40 × 4–5 µm	9–10 × 1·5–2 µm	Graddon[4]
N. indica (present study)	608–640 µ 134–192 µm	80–100 × 7–9 µm	9–20 × 3·5–5·5 µm	Authors' Collection

Being of rare occurrence and a new species addition to Indian fungi, a brief Latin diagnosis of the fungus is provided.

Niptera indica sp. nov.

Apothecia distincta, aggregate vel dispersa, cupulata vel discoidea, sessilia, superficialia, non-subiculata, atra, carnosa, haustoria immersa intro textus; magnitudine, 608–640 × 134–192 µm; excipula textura globulosa, parenchymatibus, pallide-burnnea, 50µm in diam.; epithecia nullae; asci cylindraceo vel clavatae, brevia stipitatae, unitunicatae, octosporae, magnitudine 80–100 × 7–9 µm; ascosporae distichae, ellipsoideae vel cylindracea, hyalinae, uni-septatae raro bi-vel tri-septatae, constrictae ad septa, magnit, 9·0–20·0 × 3·5–5·5 µm: parpahysoidia filiformia, hyalina et numerosa.

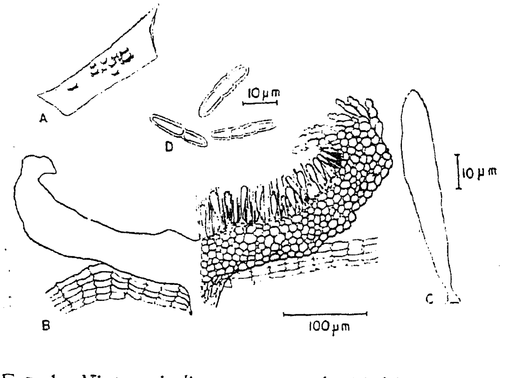

FIG. 1. *Niptera indica* sp. nov. A, Habit; B, Apothecium in V.S.; C, An ascus; D, Ascospores.

Matrix: On the wood of *Syzygium cumini* (L.) Skeels (Fam. Myrtaceae) Legit A.W.S. at Bhimashankar (2-12-1973) No. AMH 3381 (*Holotypus*).

This is a highly controversial genus with respect to its nomenclature and validity. However, recently Dennis[3] discussed its position at length and reinstated the name—*Niptera*[2]

The present collection was found to be distinct from the type species, *viz.*, *Niptera lacustris* (Fr.) Summa.[7] and the other known species in gross morphological characters besides being collected on hitherto unreported hosts.

Remarks: This fungus was also collected on *Nilgirianthus reticulatus* (Stapf.) Brem. and *Grevia hirsuta* Vahl. at Torna Fort and Mahabaleshwar No. AMH 2071, C.M.I. 197436 and No. AMH 2521, C.M.I. 193249 respectively.

Grateful thanks are offered to Prof. M. N. Kamat for his keen interest and helpful suggestions, to the Director, M.A.C.S., Poona, for laboratory facilities and to the Ministry of Education, Govt. of India, for the award of the S.R.T. to the senior author. Thanks are also due to Dr. D. N. Pegler, Royal Botanic Garden, Kew, England, for confirming the identification.

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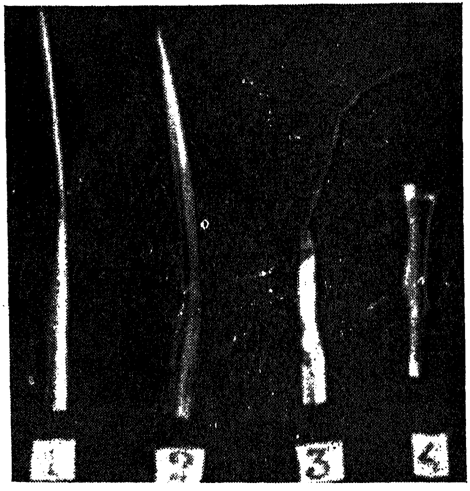
1. Ainsworth, G. C., *Dictonary of Fungi*, 1971.
2. —, Sparrow, F. K. and Sussman, A. S., *The Fungi*, 4A, Academic Press, New York and London, 1973, p. 292.
3. Dennis, R. W. G., Niptera Fr. *versus* Belonopsis Rehm. *Kew Bulletin*, 1972, 26, 439.
4. Graddon, W. D., "Discomycetes Notes and Records," *Trans. Brit. Mycol. Soc.*, 1976, 66, 169.
5. Mukerji, K. G. and Juneja, R. C., *The Fungi of India*, Emkay Publ., 1975, p. 224.
6. Rea, C., "New or Rare British Discomycetes," *Trans. Brit. Mycol. Soc.*, 1920–22, 7, 58.
7. Saccardo, P. A., *Sylloge Fungorum*, 1889, 480–486.
8. Seaver, F. J., "The Fungus Flora of St. Thomas," *Mycologia*, 1924, 16, 1.

A LEAF SPOT DISEASE OF TUBEROSE (*POLIANTHES TUBEROSA* L.) CAUSED BY A NEW SPECIES OF *ALTERNARIA*

The Tuberose (*Polianthes tuberosa*) is one of the popular commercial flowers grown in Tamil Nadu. A leaf spot disease was observed during August–December 1976 on Tuberose plants grown around Coimbatore. The disease manifests itself in the form of brown spots with faint concentric rings on the midrib and rarely on the margins of the leaf (Figs. 2–3). Occasionally spots were seen on the peduncle (Fig. 4). Infection soon leads to the drying up of the parts affected. The spots started as brown specks and attained a circular to oval shape measuring 10–30 mm in length and 4–5 mm in diameter. The number of spots per leaf varied from 1–10 and very often spots enlarged and coalesced into bigger patches. Infection on the peduncle was as dark brown patches of 10–50 mm. in length. The pathogen was a species of *Alternaria*. Pathogenicity was successfully proved by spraying spore suspensions from oat-agar cultures on healthy leaves (injured and uninjured) and it was found that a greater number of spots were produced on injured leaves than on uninjured leaves. The fungus failed to infect *Lycopersicon esculentum* Mill., *Solanum melongena* L., *Nicotiana tabacum* L., *Crossandra infundibuliformis* Nees., *Amaranthus gangeticus* L., *Capsicum annuum* L., *Jasminum grandiflorum* L., and *Chrysanthemum indicum* L.



FIGS. 1–4. Fig. 1. Healthy leaf of *P. tuberosa*. Figs. 2, 3. Infected leaves with spots. Fig. 4. Infected peduncle.

Colonies on oatmeal agar were dark brown to olivaceous. Sporulation was observed in 7–10 days-old cultures. The olivaceous to brown mycelium was septate and branched; conidiophores septate, brown to olivaceous and geniculate; conidia brown single, clavate, with a long beak, 3–7 horizontal septa and 1–2 vertical septa; conidia (with beak) measured 25·60–76·80 μ × 6·4 – 19·20 μ with a mean of 58·88 μ × 12·42 μ.

From perusal of literature it appeared that *Alternaria* has not been recorded on *P. tuberosa* so far. The characters of our fungus do not conform to any of the species of this genus so far described. Further, the failure of our fungus to infect any of the plant species other than its original host also indicated that the fungus is a new species, and it is being described as such here:

Alternaria polyanthi sp. nov.

Hypha septatis, ramulosis, olivaceo—brunnea, conidiophori septatis olivaceo—brunneae, geniculatus, conidia, singula, clavate, brunneae, rostrate, septis transversia 3–7, septis verticalibus 1–2, 25·26–76·8 μ × 6·4–19·20 μ mediet 58·88 × 12·42 μ.

Typus lectus in foliis viventibus *polianthes tuberosa* L. in Coimbatore.

Dr. J. L. Mulder, Commonwealth Mycological Institute, Kew, Surrey, England, has kindly examined the fungus and confirmed that the fungus does not belong to any of the species so far described. We are very thankful to the Director and Dr. J. L. Mulder of the CMI, Kew, Surrey, England. for this courtesy. This is also the first record of *Alternaria* on *P. tuberosa*. The fungus culture has been deposited at CMI, Kew, Surrey, England, as IMI No. 208165. The diseased leaf specimen and the culture have been deposited at the Plant Pathology Herbarium, Tamil Nadu Agriculrural University, Coimbatore (No. H.R. 12: 16).

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TUBERCULINA COSTARICANA SYD.: A NEW HYPERPARASITE ON GROUNDNUT RUST (*PUCCINIA ARACHIDIS* SPEG.)

The plants of groundnut (*Arachis hypogaea* L.) growing in Experimental Farm, Agricultural University, Jabalpur, suffered with a rust *Puccinia arachidis* Speg. during August–September 1975 and 1976. In September 1976, the hypophyllous uredia were found covered with a light purplish fungal coating. The development of uredosori and uredospores was almost checked and the whole sorus was covered by the hyperparasite.

The fungus was isolated in pure culture on potato-dextrose-agar medium from the infected rust pustules. Pathogenicity of the organism was established by spraying conidial suspension in sterilized tap-water from a 15 day-old culture in P.D.A., on a 2-month old rust uncontaminated peanut plants. The inoculated plants were incubated in polyethylene bags for 3 days. Suitable control was also maintained. The uredia were covered with a light purplish coating, producing abundant conidia in sorus within 5 days. Reisolations yielded identical culture of the organism. The uredinicolous nature of the fungus with smooth sporodochia, hyaline, amerospores confirm the genus *Tuberculina*[5] Sacc. The detailed characters are as follows:

Sporodochia light purplish, flat, extensive; conidiophores simple, nonseptate, hyaline; conidia produced acrogenously, single hyaline, smooth, thin-walled, 1-celled, slightly ellipsoid to elongate-fusiform or obovate, 7·2–10·7 × 5–5·8 μ (Fig. 1).

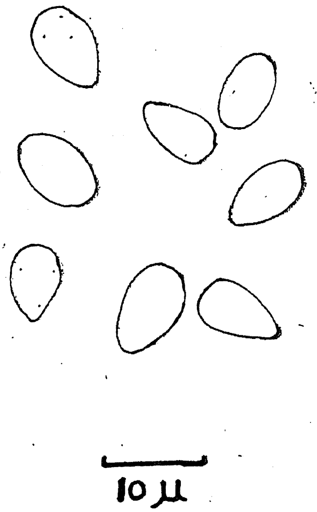

FIG. 1. Mature conidia of *T. costaricana* Syd.

On the basis of the above morphological characters of the fungus, it is identified as *T. costaricana* Sydow (*Ann. mycol. Berl.* 1927, **25**, 154). Of the two species of *Tuberculina* recorded in India[1–3,6–8], on various rusts, genus like *Phakopsora*, *Uromyces*, *Aecidium* and *Ravenelia*, only *T. persicina* (Ditm.) Sacc. parasitizes the genus *Puccinia*[4] (*P. heterospora* Berk. and Curt.). No record of *T. costaricana* on *Puccinia arachidis* is available in the literature. It is the first record of this species of hyperparasite on the genus *Puccinia* (*P. arachidis* Speg.).

The specimen has been deposited in Herb. IMI, Kew, No. 207774 and in the herbarium, Department of Plant Pathology, J.N. Agricultural University, Jabalpur.

The authors express their thanks to Dr. A. Johnston, Director and his staff of the Commonwealth Mycological Institute, Kew, for their help in the identification of the species.

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---

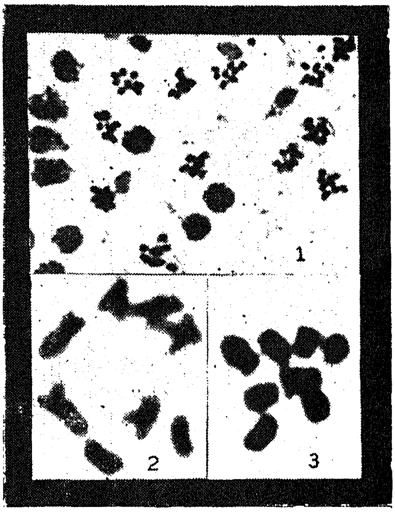
1. Butler, E. J. and Bisby, G. R., *The Fungi of India*, (Sci. Res. Monogr.), Revised by Vasudeva, R. S., I.C.A.R., New Delhi, 1960, p. 552.
2. Mukerji, K. S. and Juneja, R. C., *Fungi of India*, (1962–1972), Emkay Publ., Delhi, 1974, p. 223.
3. Rangaswami, G., Seshadri, V. S. and Lucy Channamma, K. A., *Fungi of South India*, Univ. Agric. Sci., Bangalore, 1970, p. 187.
4. Satya, H. N., *Curr. Sci.*, 1964, **33**, 148.
5. Subramanian, C. V., *Hyphomycetes*, I.C.A.R., New Delhi, 1960, p. 938.
6. Tandon, R. N. and Chandra, S., *Supplement to the List of Indian Fungi*, Univ. of Allahabad Studies, 1963, p. 246.
7. Tilak, S. T. and Rao, R., *Second Supplement to the Fungi of India*, Marathwada Univ., Aurangabad, 1970, p. 314.
8. Vasudeva, R. S., *Fungi of India, Supplement I.* (Sci. Res. Monogr.), I.C.A.R., New Delhi, 1962, p. 206.

CHROMOSOME NUMBER OF THE CESTODE *LYTOCESTUS INDICUS*

THE pausity of knowledge on the cytology of Cestodes has been traced to the technical difficulties like the small sizes of their cells and chromosomes, short duration of stages, presence of tough cuticle, powerful muscles and the syncytial nature of parenchymatous cells rendering the material arduous for squashing[1]. Among the 37 genera and 89 species of Caryophyllidea only few a members such as *Archigetes sp.*[2], *Hunterella nodulosa*[3], *Atractolytocestus huronensis*[4], and *Glaridacris laruei*[5] have been studied for their chromosome numbers. The genus *Lytocestus* belonging to the subfamily Lytocestinae and possessing seven species[6,7] has not been investigated for its cytology. The chromosome number of *Lytocestus indicus* is reported here.

The parasites collected from the fresh water fish *Clarias batrachus* were washed in Ringer's A solution[8] and treated for 2 hours at room temperature in the same solution containing 1 part of 0·05% colchicine to 5 parts of the former. Material was fixed in acetic alcohol and processed by the haematoxylin squash method described elsewhere[9] by a slight variation of using the mid regions of parasites containing the testes instead of the entire parasites for processing. The testes were teased before squashing in a drop of 45% acetic acid on a slide employing a binocular microscope.

A lack of divisional figures in sufficient numbers was noticed in the material collected from the hosts at different time intervals. It has been shown in *Diphyllobothrium dendriticum*[10] that starvation of the host reduces the mitotic activity and that nutrients keep the same at a higher level. It is not known whether the absence of divisions described here is due to a similar situation. In instances where divisions were found, they were seen in abundance. Difficulty was also experienced in getting good spreads of chromosomes enabling accurate counts. Hence a large number of cells were scored for the purpose. Figure 1 shows a group of cells in meiotic division. That sixteen chromosomes constitute the diploid number of *L. indicus* is evident from the presence of eight bivalents in diakinesis (Fig. 2) and metaphase I (Fig. 3). Detailed analysis of mitotic chromosomes from this as well as other species of these otherwise fastidious parasites is being envisaged with a view to constructing caryotypes and idiograms by standard methods and discuss their interrelationships from systematic and evolutionary points of view.



FIGS. 1-3. Haematoxylin squashes of testes of *L. indicus*. Fig. 1. A group of cells in meiotic division. × ca. 700, Fig. 2. Diakinesis, × ca. 2,500. Fig. 3. Metaphase I. × ca. 2,500.

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1. Wikgren, B. J. P. and Gustafsson, M. K. S., *Acta Acad. Aboensis*, 1965, **25**, 1.
2. Motomura, L., *Annot. Zool. Japanenses*, 1929. **12**, 109 (Cited by Grey and Mackiewicz, 1974),
3. Mackiewicz, J. S. and Jones, A. W., *Proc. Helminthol. Soc. Washington*, 1969, **36**, 12.
4. Jones, A. W. and Mackiewicz, J. S., *Jour. Parasitol.*, 1969, **55**, 1105.
5. Grey, A. J. and Mackiewicz, J. S., *Exptl. Parasitol.*, 1974, **36**, 159.
6. Mackiewicz, J. S., *Ibid.*, 1972, **31**, 417.
7. Rama Devi, P., *Jour. Helminthol.*, 1973, **67**, 415.
8. Darlington, C. D. and La Cour, L. F., *The Handling of Chromosomes*, George Allen and Unwin Ltd., London, 1966.
9. Venkat Reddy, P. and Subramanyam, S., *Curr. Sci.*, 1971, **40**, 578.
10. Wikgren, B. J. P., Knuts, G. M. and Gustafsson, M. K. S., *Zeit. Parasitenk.*, 1970, **34**, 242.

SUPERNUMERARY SPIKELETS IN BREAD WHEAT

ONE spikelet at a point on the rachis is a normal condition in wheat. Varieties like NP 863 may have two spikelets at some points[1]. Genes for suppression of spikelet reduplication are already known to be associated with the left arm of chromosome 2A and the right arm of chromosome 2D[2].

Eleven F_2 populations monosomic for chromosomes; 1A, 1B, 1D, 2D, 3D, 4A, 4D, 5D, 6A, 6D and 7B, were compared with normal, disomic F_2 population of Red Bobs × Sharbati Sonora. 19 plants with supernumerary spikelets and (or) additional leathery florets (Fig. 1) were found in the F_2 population derived from monosomic F_1 for chromosome 7B which consisted 203 individuals. These were the only plants affected in a total of 1,800 plants observed from all the populations. The number of positions, at which these spikelets appear, varied within and between the

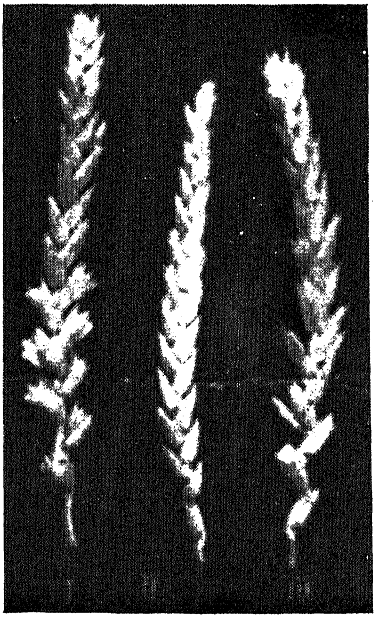

FIG. 1. (i) Spike with supernumerary spikelet and florets. (ii) Normal spike. (iii) Spike with supernumerary spikelets.

plants. Uusually, the first tiller had greater number of such spikelets than those in the late tillers. Two such plants on cytological observation were found to be monosomic (41 chromosomes). It is obvious that all the monosomic plants did not possess supernumerary spikelets.

It is important to note that Red Bob monosomics for chromosome 7B have a tendency to bear supernumerary spikelets at one or two positions on the spike. Penetrance of this character appears to be incomplete. Further, supernumerary spikelets were not found in F_1 monosomic for 7B.

These observations indicate that chromosome 7B is associated with the formation of normal spikletes. Monosomic condition for chromosome 7B itself is sufficient to produce abnormal spikes in Red Bobs. But this is not true with chromosome 7B of Sharabat i Sonora as evident in the monosomic F_1 where the univalent is from Sharbati Sonora. The appearance of this character again in F_2 progeny of monosomic F_1 indicates genes on other chromosomes too. The expression of the character under consideration, thus, required segregation of favourable alleles together with monosomic condition for chromosome 7B. Thus the Monosomic F_2 analysis of Red Bobs × Sharbati Sonora for eleven chromosomes revealed the association of genes for normal spikelet with chromosome 7 B.

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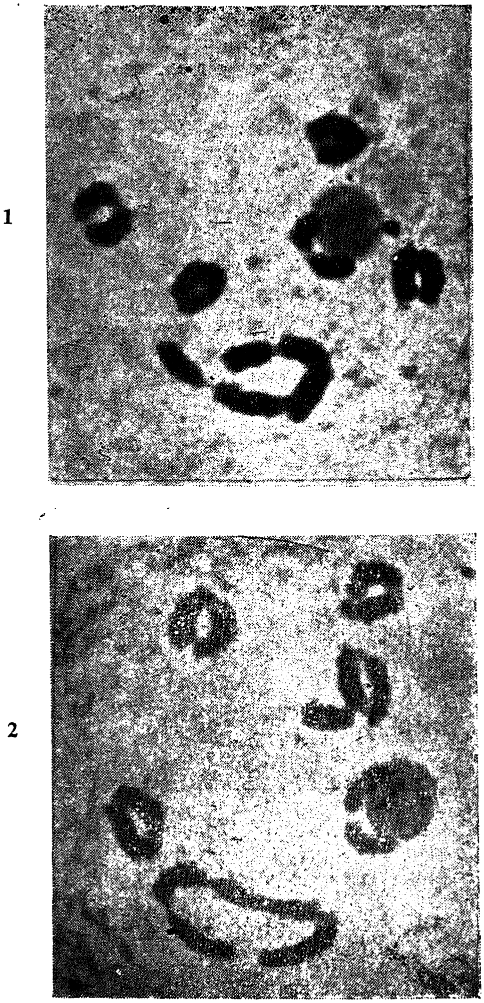
1. Goud, J. V., *Indian J. Genet.*, 1967, **27**, 40,
2. Sears, E. R., *Univ. Mo. Agr. Exp. Sta. Res. Bul.*, 1954, **572**, 59.

TWO INTERCHANGE TRISOMICS IN PEARLMILLET

IN pearlmillet, primary trisomics were grouped into morphologically distinct types by Gill, Virmani and Minocha[1] and Manga.[2] One type designated as Slender trisomic by both the authors was reported by the latter to have produced on selfing an unrelated primary trisomic. In the fourth generation progeny of this Slender trisomic two of the plants obtained on open pollination were morphologically different from control diploids. One was of the 'Slender' type and the other resembled to some extent the 'Bush' type trisomic described by Manga[2]. The Bush trisomic corresponds to the Dark green trisomic described by Gill *et al.*[1] The 'Bush' type plant differed from the typical Bush or Dark green trisomic in that it had narrow, stiff and erect leaves, with 8 tillers and a few lateral branches and was very late flowering.

Study of meiosis in pollen mother cells revealed that both plants were trisomics, with $2n = 15$ chromosomes and in both associations of five chromosomes wer observed (Fig. 1). 500 PMC's in the 'Slende type plant and 100 PMC's in the 'Bush' type pla were examined at diakinesis and the data relating the types of associations observed are presented Table I.



FIGS. 1–2. Two PMC's from an interchange trisom
Fig. 1. Showing an association of five chromosom
Fig. 2. Showing a ring of four chromosomes ar
an unpaired chromosome.

It can be seen from the table that associations 5 chromosomes were frequent. An association five chromosomes in a trisomic is possible under tw conditions: (1) when the extra chromosome is a te tiary one and (2) when an extra chromosome, whic is a normal one, is present in a heterozygous inte change background. In the latter case when the ext chromosome remains as a univalent a ring of fo

TABLE I

Chromosome associations at diakinesis in the 'Slender' and 'Bush' type plants

Chromosome association	Proportions of cells in each class in %	
	'Slender'	'Bush'
1 v + 5 ii	38·0	32·0
1 iv^{c} + 5 ii + 1 i	12·6	10·0
1 iv^{r} + 5 ii + 1 i	11·8	6·0
1 iii + 6 ii	29·6	39·0
1 iii + 5 ii + 2 i	2·0	2·0
6 ii + 3 i	0·4	2·0
7 ii + 1 i	5·6	9·0
No. of cells examined	500	100

c — chain of four. *r* — ring of four

configurations is expected[3]. As can be seen from the table rings of four chromosomes were found in both plants (Fig. 2). From this it is concluded that both the plants were interchange trisomics.

One of the methods of assessing preferential pairing is studying the frequency of trivalents and closed bivalents formed by the interchange complex[4]. In the present study it was observed that this condition was at a low frequency; 8% of the PMC's in the 'Slender' type plant and 14% of the PMC's in the 'Bush' type plant contained such a configuration. Hence it was concluded that preferential pairing was not of significance in the present interchange trisomics.

We are thankful to Dr. J. V. Pantulu for guidance and to the U.G.C. and the C.S.I.R. for the award of research fellowships.

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1. Gill, B. S., Virmani, S. S. and Minocha, J. L. *Can. J. Genet. Cytol.*, 1970, **12**, 474.
2. Manga, V. *Ph.D. Thesis* submitted to the Andhra University, Waltair, 1972.
3. Burnham, C. R., *Discussions in Cytogenetics*, Burgess, Minnesota, 1962, p. 146.
4. Sybenga, J., *Genetica*, 1966, **37**, 481.

A NEW SPECIES OF *MELAMPSORA* FROM ANDHRA PRADESH

RECENTLY Ramachar and Bhagyanarayana[3] described *Caeoma euphorbiae-geniuclatae* a rust fungus parasitizing on *Euphorbia geniculata* Ortega from Hyderabad. In their paper the reason as to why *Melampsora euphorbiae-geniculatae* previously reported by Kern and Thurston[1] also on *Euphorbia geniculata* as *sed illegit* was pointed out. Since then the authors made intense search for the other spore forms produced by this rust fungus and succeeded in finding out the telia which is described in this paper.

Melampsora geniculatae Ramachar and Bhagyanarayana sp. nov. (Fig. 1.)

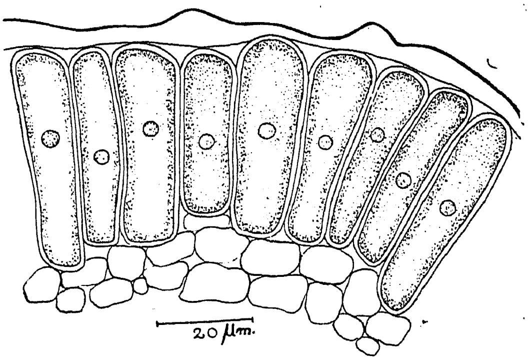

FIG. 1. *M. geniculatae*, vertical section of a *Telium*.

M. euphorbiae-geniculatae Knern and Thurston, *Mycologia* **36**: 507, 1944, nomen valid, sed illegit.

Caeoma euphorbiae-geniculatae Ramachar and Bhagyanarayana, *Trans. Br. mycol. Soc.* **67** (1): 139–140, 1976.

Spermagonia ignotis. Aeciis amphegenis, pleurmque hypophyllis, caulicolis, in areas magnus plus minus uniformiter dispersis, aggregatis vel confluentibus in maculis 0·5–1·5 mm diam. flavescentibus, dein pulverulentis, paraphysibus, epidermide rupta conspicua; sporis ellipsoides vel globosis, 12–15 (–16·5) × 10–13·5 µm, membrana 1–2 µm crassa, hyalina vel flavida, minute verrucosa. Uredinis ignotis. Tellis minutes, hypophyllis, non visis, subepidermalibus; sporis 27–54·4 x 16 µm, cylindraceis, palide aureobrunneis, membrana ad apicen. et ad latere 1 µm crass.

Spermagonia not known. Aecia caeomoid, amphygenous, usually hypophyllous yellowish, pulverulent, paraphysate; aeciospores 12–15 (–16·5) × 10–13·5 µm, ellipsoidal or globoid, wall 1–2 µm, thick, hyaline or yellowish, minutely verrucose. Uredinia not known. Telia subepidermal, hypophyllous, minute; teliospores 27–54·4 × 9–16 µm, cylindrical pale golden brown, uniformly 1 µm, thick.

Holotype: On *Euphorbia geniculata* Ortega, Botanical Garden, Osmania Univesity, Hyderabad, June 1976, Ramachar and Bhagyanarayana. The type slide has been deposited at C.M.I.

Melampsora geniculatae appears to be a microcyclic and autoecious rust fungus. The distinct features of this species is the production of only two spore forms in its life cycle, *viz.*, aeciospores and teliospores. Petersen[2] classifies this type of life cycle under catopsis type,

The authors wish to express their thanks to Prof. Jafar Nizam, Head of the Department of Botany, Osmania University, for his encouragement. The help given by Drs. N. Ramayya and Miss B. K. Vaidehi, Readers in Botany, Osmania University, is appreciated. One of us (G. B.) is grateful to the Osmania University authorities for awarding a U.G.C. Junior Research Fellowship.

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---

1. Kern, F. D. and Thurston, H. W. Jr., *Mycologia*, 1944, 36, 507.
2. Petersen, R., H. *Bot. Gaz.*, 1974, 40 (4), 456.
3. Ramachar, P. and Bhagyanarayana, G., *Trans. Br. mycol. Soc.*, 1976, 67 (1) 139.

LEVEL OF GLUTAMIC AMINOTRANSFERASE AND GLUTAMIC DEHYDROGENASE ENZYMES IN SEED

THE presence of two important enzymes in nitrogen metabolism, *viz.*, glutamate-oxaloacetic aminotransferase (GOT; E.C. 2.6.1.1) and glutamate dehydrogenase (GDH; E.C. 1.4. 1.3) have been reported in seeds[1,2]. It has been shown that the activities of these enzymes, present in minute amounts[3], vary to a considerable extent in seeds and seedling[4]. It probably depends on the type of seeds with respect to the ability to activate the existing enzyme or *de novo* synthesis of enzyme protein during the germination process. There is no report in the literature to show whether there is any relation between concentration of the protein with the levels of these enzymes in mono and dicot seeds. In the present experiment, an attempt has been made to study this.

Seeds of wheat (var. NP 876), barley (var. J rice (var. BC–6), mustard (var. T 39), pea (var. mung (var. PS 16), soybean (var Glycine max tobacco (var. Virginia) were selected for the Five g of the seed powder was macerated with of Tris-HCl (0·2 M. pH 8·2 containing triton). Cell debris were removed through layers of muslin cloth and the filtrate was fuged at 25,000 × g for half an hour at 5° C. supernatent was dialysed at 5° C against the buffer for 18 hours and the precipitate formed dialysis was removed by centrifuging at 2,000 pH of the extract was adjusted to 8·2 and 8·6 for and GOT enzymes, respectively. The assays of the enzymes were carried out following the me described earlier[2]. The specific activity of the en is defined as the amount of product formed reaction mixture per min per mg protein in the extract. The soluble protein in the enzyme was determined following the methods of *et al.*[5]. The protein content of the seed was mined on the basis of nitrogen in the digested ma

Results on enzyme activity and the protein of seed are presented in Table I. It is observ wheat, barley and rice have higher level of the enzymes than the dicots studied. The highe of enzymes was recorded in wheat. It m mentioned that the high amount of seed protei related with the amount of above two enzymes the other hand a low activity of the enzymes recorded. Further GOT and GDH activitie not related to each other at least in ungerm seed. However, it is possible that they may d some relation during the germination process later part of growth since both the enzymes a group closely associated to nitrogen metaboli this deserves further investigation.

TABLE I

Protein content, glutamate-oxaloacetic aminotransferase and glutamate dehydrogenase activities in seed Triplicate samples were taken and the values given (± SE) are mean of three observations

Seed	Protein content % dry weight	GOT activity *n* moles oxaloacetate formed/min/mg protein	GDH activity *n* moles NADH oxidized/min/mg protein	GOT/GDH
Wheat	12·2±0·24	194·4±15·5	2·42±0·14	81
Barley	12·8±0·25	54·3± 3·2	1·44±0·05	37
Rice	7·3±0·14	56·6± 3·4	1·12±0·05	50
Pea	22·0±1·12	13·2± 0·5	0·49±0·01	27
Mung	23·6±1·18	59·2± 3·5	0·76±0·03	78
Soybean	40·2±2·01	42·6± 2·4	0·91±0·04	47
Mustard	25·3±0·75	4·9± 0·1	0·68±0·05	7
Tobacco	33·1±1·32	39·4± 1·9	0·94±0·03	42

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1. Smith, B. P. and Williams, H. H., *Arch. Biochem. Biophys.*, 1951, **31**, 366.
2. Srivastava, G. C. and Fowden, L., *J. Exp. Bot.*, 1972, **23**, 921.
3. Doby, G., *Plant Biochemistry*, Interscience Publishers, 1965, p. 484.
4. Leonard, M. J. K. and Burris, R. H., *J. Biol. Chem.*, 1947, **170**, 201.
5. Lowry, O. H., Rosenbrough, N. J., Farr, A. I. and Randall, R., *Ibid.*, 1951, **193**, 265.

RADIATION INDUCED AMBER-GRAINED MUTANTS IN VARIETY TONARI 71 OF WHEAT

THE value of induced mutagenesis in plant breeding has been a point of debate for a considerable time and the opinions have differed from euphoria at one extreme to complete pessimism on the other. It has, however, now come to be accepted that for rectification of simply inherited shortcomings of varieties otherwise agronomically very superior, mutation breeding is a very useful adjunct to the conventional plant breeding procedures. In this communication we report mutations for grain colour induced by gamma-ray treatment in the variety Tonari 71. Tonari 71 was released from the CIMMYT programme in Mexico in 1971 and has the advantage of high yield, high protein content (14·9%) high sedimentation value (45·2 cc) and a high degree of field resistance against all the three rusts. In the special varieties category of the All India Coordinated Wheat Trials, Tonari 71 recorded the highest yield in the year 1972-73 and was characterized by a very wide adaptability. This variety however, has the handicap of possessing red coloured seed which lowers its consumer acceptability.

Dry seeds of Tonari 71 were treated with 30 KRad of Co^{60} gamma-ray in one experiment. In another experiment, seeds presoaked for 17 hours in water were treated with 0·02% nitroso methyl urea (NMU) for six hours at 25° C. The seeds were sown in the fields for raising M_1 plants. The M_1 plants were selfed to eliminate any outcrossing and thrashed individually with care to prevent any mechanical mixture. Large M_2 populations were grown for both the treatments and rigorous screening was exercised for plants with amber coloured grains. The NMU treatment did not yield desired results but one plant with amber grains was identified among the M_2 population from the gamma-ray treatment. This plant was identical with the parent variety with the exception that it has colourless auricles and waxy earheads and leaves instead of pink auricles and non-waxy head and leaves. The mutant had also a tendency to be late in maturity by about six days. Progenies of the 29 selected mutant plants bred true for amber grain in the M_3 and subsequent generations. Seeds from single plant progenies of these 29 plants were bulked in M_4 to compare the yield performance of the mutant with the parental variety. Data were also recorded on yield contributing attributes such as average productive tillers per plant, the average number of grains per earhead and thousand kernel weight. This information is smmarised in Table I. It is observed that

TABLE I

Data on yield and some yield contributing attributes on selected amber grain mutants of Tonari 71

Mutant line	Grain yield (kg) three 15′ rows	Number of productive tillers/plant (Av. of 5 plants)	Number of grains per panicle (Av. of 5 plants)	Weight of 1000 kernels (gm)
Tanori 71 Control	1·35	9·0	73·55	38·5
TM–1–2	1·50	16·4	59·6	42·0
–4	1·50	17·0	65·2	43·0
–11	1·50	11·4	64·0	44·0
–14	1·60	11·8	54·0	44·0
–16	1·70*	12·2	55·0	41·0
–20	1·75*	8·2	57·6	42·5
–23	1·55	12·2	60·1	42·0
–24	1·50	15·4	67·0	42·0
–25	1·50	11·0	57·0	43·0
–28	1·70*	10·4	57·2	43·5
–29	1·50	9·2	52·0	42·0
–30	1·50	14·2	60·4	42·0
–36	1·75*	10·2	53·0	43·0
–40	1·50	12·6	61·6	45·0
–44	1·65	12·8	65·0	43·5
–49	1·40	15·2	62·0	44·0
–54	1·50	10·8	71·0	43·0
–73	1·50	9·8	55·2	44·0
–77	1·50	10·0	58·4	43·0
–83	1·60	12·8	62·0	40·0
–84	1·50	13·8	57·0	44·0
–87	1·50	8·7	56·8	44·0
–90	1·80*	8·3	57·0	40·0
–93	1·60	9·2	57·6	43·0
–97	1·55	9·2	62·4	44·0
–100	1·70*	11·2	60·4	45·0
–101	1·60	9·5	53·2	42·0
–102	1·50	12·2	54·6	45·0
–107	1·50	10·4	60·0	42·0
SE_m	0·106	2·366	5·146	1·524
C.D.at 5%	0·306	6·843	14·883	4·422

a degree of inter-culture variability is present for all the attributes studied. This is not surprising when one considers that radiation treatment will randomly affect a number of loci and thus produce genetic heterogeneity in the off-spring. Another point of interest is that the data on selected promising progenies clearly indicate that in the matter of yield the mutants are atleast at par with, or even superior to, the parental variety.

Mutagenically induced amber grain mutants have been reported previously.[1,2] The realisation in mutation experiment of a desired grain colour rectification of Tonari 71 underlines the value of mutation breeding as a tool when applied to well planned experiments with limited objectives.

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---

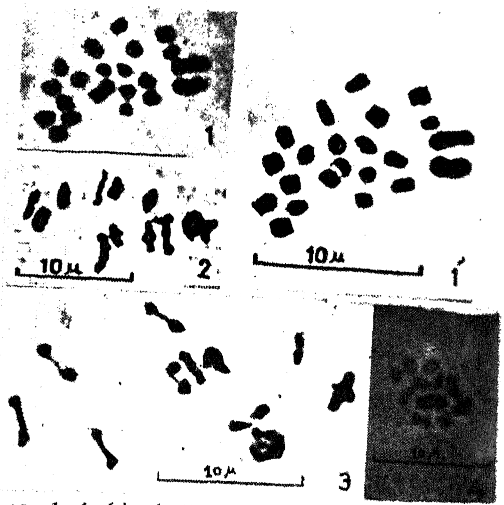
1. Verghese, G. and Swamlnathan, M.S., *Cnrr. Sci.* 1966, **35**, 469.
2. Sawhney, R. N., Jain, H K., Joshi, B. C. and Singh, D., *Proc. Int. Symp. on use of Isotopes and Radiation in Agriculture and Animal Husbandry Research*, New Delhi, 1973, p. 214.

CYTOGENETICS OF COMMELINACEAE: VI. A New Basic Chromosome Number for the Genus *Floscopa*

Differing chromosome numbers have been reported by earlier workers for *Floscopa scandens* Lour. as n or $x = 9^1$, 12^{2-4} and 15^5. We are reporting here the number of $2n = 22$ and $n = 11$, which is new for this species and also for the genus. In a population of plants, two types differing only by poor and vigorous vegetative growth occurred in Taukucha-Rangtia forest and also in Sylhet of Bangladesh. The plants were brought to Dacca and grown at the Dacca University Botanical Garden. Cytological observations were made for three successive years from the same population.

In studying the chromosomes of root tip cells. haematoxylin squash method[6] preceded by pretreatment in saturated paradichlorobenzene for three hours was used. Acetocarmine squash method[7] was used to study meiosis in the pollen mother cells.

The root tip cells from both types showed 22 chromosomes (Figs. 1 and 1′). The chromosomes were heteromorphic with one pair being much larger than the rest. The pollen mother cells of both types contained 11 bivalents (Figs. 2, 3 and 4). The mean chiasma frequency per bivalent estimated from 36 well-spread pollen mother cells. along with standard error ($\bar{X} \pm$ S.E.), was $1 \cdot 95 \pm 0 \cdot 03$. Regular rence of 22 chromosomes in root tip cells as well bivalents in pollen mother cells indicated that basic number was 11. This record seems to su Brückner's[8] classification of this genus under the *Commelineae* and Brenan's[9] categorization his "group I" where several members possess chromosomes.



Figs. 1–4. Fig. 1. Somatic chromosomes in tip cell; Fig. 1′, Camera lucida drawing of Figs. 2, 3 and 4. 11 bivalents in pollen mother

We thank Dr. D. K. Banerjee, Deputy Dir Botanical Survey of India and Dr. M. S. Khan, ment of Botany, University of Dacca, for ther in the identification of the plant.

Department of Botany, M. A. Zaman.
University of Dacca, B. N. Chakra
Dacca 2, Bangladesh.
April 12, 1977.

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1. Morton, J. K., *J. Linn. Soc. Lond.* (*Bot.*), 60, 167.
2. Kammathy, R. V. and Rolla, S. R., *Bull Surv. India*, 1961, **3**, 393.
3. Rolla, S. R., Kammathy, R. V. and Rag R. S., *J. Linn. Soc. Lond.* (*Bot.*), 1968,
4. —, Raghavan, R. S. and Kammathy, *Bull. Bot. Surv. India*, 1970, **13**, 242.
5. Bhattacharya, B., *Cytologia*, 1975, **40**, 2
6. Aziz, Mia, M. A. and Mansur, M. A., *Science and Applications*, 1972, 6, 23.
7. Sharma, A. K. and Sharma, A., *Chro Technique, Theory and Practice*, 1st ed., worths and Co. (Publishers) Ltd., 196
8. Brückner, G., *Dia Natural. Pflanzenfam.*, A. S. and Prantl, K., 2nd ed., 1930, 15
9. Brenan, J. P. M., *J. Linn. Soc. Lond.* (*Bot.* **59**, 349.

SHORT SCIENTIFIC NOTES

Some Additions to Indian Ascomycetes

A number of ascomycetous fungi were collected in the year 1976 during survey of ascomycetous fungi of Bhagalpur, Bihar. They were sent to C.M.I., Kew, England, for identification. Out of these five were found to be unrecorded from India. So they are listed below with their hosts and locality. This is the first report of their occurrence in India.

1. *Clathridium corticola* (Fckl.) Shoemaker and Muller (IMI–209198).
   On dead twigs of *Clitoria ternatea* L. from Botanical Garden, Bhagalpur University, Bhagalpur.
2. *Ophiobolus spirosporus* Ahmad (IMI–207754).
   On submerged and living leaves, leaf sheaths and stems of *Saccharum spontaneum* L., Zoological tank, Bhagalpur University, Bhagalpur.
3. *Didymosphaeria oblitescens* (Berk and Br.) Fckl. (IMI–209197).
   On dead twigs of *Premna mucronata*, Botanical Garden, Bhagalpur University, Bhagalpur.
4. *Lophiostoma vagans* Fabre. (IMI–207751).
   On dead twigs of *Jasminium sambac* Ait., Botanical Garden, Bhagalpur University, Bhagalpur.
5. *Lophiostoma angustilabrum* (Berk and Br.) Cke (IMI–209205).
   On dead twigs of *Solidago* sp., Botanical Garden, Bhagalpur University, Bhagalpur.

The author expresses his gratefulness to the Director, C.M.I., Kew, England, for identification and to Professor K. S. Bilgrami, Head, P.G. Department of Botany, Bhagalpur University, for necessary laboratory facilities.

P. G. Botany, M. A. RIZWI.
Bhagalpur University,
Bhagalpur 812 007,
February 12, 1977.

Diplodia carissae Ahmad on *Carissa*—A New Record for India

During November 1973 a severe leafspot disease was observed at Khargone on the foliage of the *Carissa carandas* Linn. The synptoms were observed only on leaves. Both the surfaces of affected leaves bore irregular, necrotic spots, measuring several cm. in diameter and covered almost half of the leafblade. The fungus was identified as *Diplodia carissae* Ahmad. Repeated isolations of the fungus on Potato-Dextrose-Agar (P.D.A.) confirmed the association of *Diploidia carissae* Ahmad with the lesions. The herbarium material has been deposited under IMI No. 177822. *Diplodia carissae* is a new fungus record for the country[1–3] and *Carissa carandas*, a new host record.

The author is grateful to Dr. Punithalingam of Commonwealth Mycological Institute, Kew, England, for confirming the identity of the fungus and to Dr. G. P. Agarwal, Head, Department of Postgraduate Studies and Research in Botany, University of Jabalpur, Jabalpur, for encouragement and advice.

Department of Botany, R. C. RAJAK.
Government Science College,
Jabalpur, *August* 25, 1976.

1. Butler, E. J. and Bisby, G. R., *The Fungi of India* (Revised by R. S. Vasudeva), I.C.A.R., New Delhi, 1960, p. 512.
2. Tandon, R. N. and Chandra, S., *The Fungi of India, Supplement to the List of Indian Fungi*, University, Allahabad Studies, Allahabad, 1963 p. 246.
3. Mukerji, K. G. and Juneja, R. C., *Fungi of India, Supplement to the List of Indian Fungi*, Emkay Publications, Delhi, 1975, p. 224.

Fungi Associated with Rai (*Brassica juncea* Hook. f. & Thoms). Seeds

Rai is economically important crop, the seeds of which are used as condiments and in various medicines. In the present investigation, therefore, it has been intended to study the fungi associated with rai seeds, which play a vital role in biodeterioration of seeds.

For the surface mycoflora, the surface washings (washed the seed surface sterilized with double distilled water) of unsterilized seeds were plated on blotter paper, Czapeck's-dox-agar and potato-dextrose-agar media separately. For internal mycoflora, the seeds were sterilized (with $0 \cdot 1\%$ $HgCl_2$ solution) and plated on above media separately as followed by ISTA (Anonymous, 1966)[1]. All the plates were incubated at 25 ± 2° C for a week. Of the 17 fungal ioslates obtained in this way *Aspergillus flavus* Link ex Fries, *A. niger* Van Tieghem *A. tamarii* Kita, *A. terreus* Thom., *Alternaria* sp., *Cladosporium herbarum* (Pers.) Link and *Penicillium* sp. isolate No. 1 were found associated both ecto and endophytically. *Aspergillus flavus*, *A. luchuensis* Inui, *A. sulphureus* (Fres) Wehmer, *A. versicolor* (Vuill.) Tiroboschi, *Alternaria tenuis* Nees (Sacc), *Cladosporium herbarum*, *Cunninghmella* sp., *Curvularia* sp., *Fusarium semitectum* Berkeley and Ravenel, *Fusarium* sp., *Helminthosporium* sp. and

Penicillium sp. isolate No. 2 were found only in endophytic association.

The isolates were identified by comparing their characteristics with those of the stalk cultures maintained at the Department and also comparing them with the type descriptions available.[2–4]

Plant Pathology Research Laboratory,
Department of Botany,
Agra College, Agra 282 002,
August 30, 1976.

Y. K. GUPTA.
V. K. AGARWAL.
A. N. ROY.
M. N. GUPTA.

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1. Anonymous, *Proc. ISTA*, 1966, 31, 1.
2. Barnett, H. L., *Illustrated Genera of Imperfect Fungi*. Burgess Publishing Co., 426 S. Sixth Street, Minneapolis 15, Minn. (Second edition). 1960.
3. Gilman, J. C., *A Manual of Soil Fungi*. The Iowa State College Press, Ames., Iowa, 1945.
4. Subramanian, C. V., *Hypomycetes*, ICAR Publication, New Delhi, 1971.

Two New Diseases of Grape Berries

Phoma macrostoma Mont. and *Cladosporium tenuissimum* Cooke were found to be associated with the decay of grape berries (*Vitis vinifera* L.) in storage and transit at Allahabad. The decay caused by both the fungi appeared through the stalk-end or growth cracks on the fruits. Pycnidia of *P. macrostoma* and cottony growth of *C. tenuissimum* were observed on the infected berries. No single fruit was found to be infected simultaneously by both the organisms. The losses caused by *P. macrostoma* as well as *C. tenuissimum* varied 2–6%. Wangikar *et al.*[1] recorded *Cladosporium oxysporum* on grape berries, but the above organisms have not been reported on fruits of *V. vinifera* from India.

The author is thankful to the Director, C.M.I., Kew, England, for confirming the identity of both the organisms (No. IMI, 199042 and 199041 respectively).

P.G. Department of Botany,
Bhagalpur University,
Bhagalpur-7, *October* 28, 1976.

JAMALUDDIN.

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1. Wangikar, P. D., Raut, J. G. and Gopalkrishnan, N., 1969, 22, 403.

REVIEWS AND NOTICES OF BOOKS

Annual Review of Phytopathology (Vol. 14), Editor: K. F. Baker, Associate Editors: G. A. Zentmyer and E. B. Cowling (Annual Reviews, Inc., 4139, Elcaminoway, Paloalto, California 94306, U.S.A.), 1976. Pp. 1–511, 22 × 14·5 cms. Price: $ 17.00, Elsewhere $ 17.50.

This 14th volume in the series includes a prefatory chapter followed by Historical perspectives, Appraisal of plant disease, Pathogens, Morphology and Anatomy, Physiology of host-pathogen interaction, Genetics of host-pathogen interaction, Epidemiology, Influence of environment, Chemical control, Biological and cultural control, Breeding for resistance, Special topics, finally ending with the Indexes. The prefatory chapter which consists of four essays by Vanderplank whose photograph forms the frontispiece are particularly interesting and thought provoking. An important additional feature of this volume is a chapter on special topics which includes the plant disease clinic, fossil fungi and electrophysiological research in plant pathology. Though information on fossil fungi may not appear significant, a careful study of this topic shows, as rightly concluded by Pirozynski, that the fossil fungi can serve a potential as interpreters of past environments and this will be realised only after a coordinated study of fossil and modern fungi is undertaken.

As usual, the various chapters in this volume have been extremely well written by specialists in the field and as rightly put forward in the preface, the *Annual Review of Phytopathology* has definitely served in the analytical synthesis of an expert to highlight significant progress and to show the remaining gaps.

Finally, Dr. Vanderplank while concluding his four masterly essays (p. 10) effectively points out that "The twin features of science, the publication explosion and ever-increasing specialization are going to make us need the *Annual Review* more and more". The present volume thus amply justifies the statement. This volume without hesitation must find a place in all institutions where phytopathologica studies are carried out.

K. SUBRAMANYAM.

Annual Reviews of Physical Chemistry, Vol. 27 (Annual Reviews Inc., California), 1976, Pp vii + 630. Price: USA $ 17.00, Elsewhere $ 17.5

Annual Reviews of Physical Chemistry has bee a valuable publication of high quality since its ince tion. The present volume is no exception. This i first volume where Professor Henry Eyring is not editor, but the patriarch has contributed an article the theory of water structure. Of the total of 22 cles in the volume, eight are related to rate theory kinetics and twelve broadly come under structure thermodynamics. We shall first examine the a of kinetics.

he history of H_3 kinetics has been reviewed by hlar and Wyatt, restricting their attention to atomic ange, dissociation and recombination reactions. elopments in the study of Molecular dynamics by nte Carlo methods (since Alder's review in 1973) been reviewed by Wood and Erpenbeck. Kine- of nucleation and growth of crystals have been nined by Kahlweit while recent developments in theory of isolated polymer molecule, dynamics in ion and associated viscoelastic properties form hapter by Bixon. Molecular motions including effects, formation of encounter complexes, motions olvent molecules and conformational changes of cules have been discussed as factors affecting tion rates by Grunwald, Chang and Leffler. There article by King and Setser on gas phase electronic gy transfer processes of excited atoms with sufficient energy to yield electronically excited products ncounters with other atoms or molecules and her on the simplest type of energy transfer colli- process (conversion of translational into internal tional and vibrational energy) by Toennies. The le on chemical reactions at very low temperatures oldanskii introduces an area of potential experi- tal possibilities.

the area of structure and spectroscopy, there are w articles on spectroscopy of simple free radicals ler), resonance Raman effect (Johnson and Peti- s) and the new area of depolarized light scattering its application to study of reorientation of small cules, intermolecular interactions, orientational lations, liquid crystals and dynamics of macro- cules (Bauer, Brauman and Pecora). The article esonance Raman deals with large organic mole- s in condensed phase and the article on free cal spectroscopy is limited to closed shell species h are clearly chemical fragments. Schaefer's le on the theory of molecular electronic structure rs important and innovative contributions since is's review of 1973. Recent advances in protein allography have been described by Matthews. ther review in this volume which includes spectroscopic and structural studies is the one by Gole on recent developments in high temperature chemistry; this article includes, amongst other topics, single collision and low-pressure chemiluminescence studies. Transport of electronic excitation from molecule to molecule in crystals and its effect on EPR spectra and fluorescence have been discussed by Silbey. The review on Van der Waals molecules in gas phase by Blaney and Ewing is interesting and includes preparation, detection, structure, properties, and so on, of these short-lived species.

The article by Gasparoux and Prost on liquid crystals covers various aspects, particularly the elastic and hydrodynamic properties as well as phase transitions. On phase transitions beyond the simple Ising model, there is a brief article by Nagle and Bonner who discuss modern developments like multitern systems, multicritical points, lattice systems with hard interactions and intermediate models. There is an interesting article on spread lipid films on water by Gershfeld who discusses thermodynamics, intermolecular forces and dynamic properties besides reactions in surface films. The article by Swinton reviews recent developments in the traditional area of solutions of non-electrolytes. An article on pyrometallurgy by Kellogg, Rao and Marcuson is also included in this volume. While the nature of this article is distinctly different from others, it is probably a good idea to have one or two such articles related to materials preparation and processes in a volume on physical chemistry.

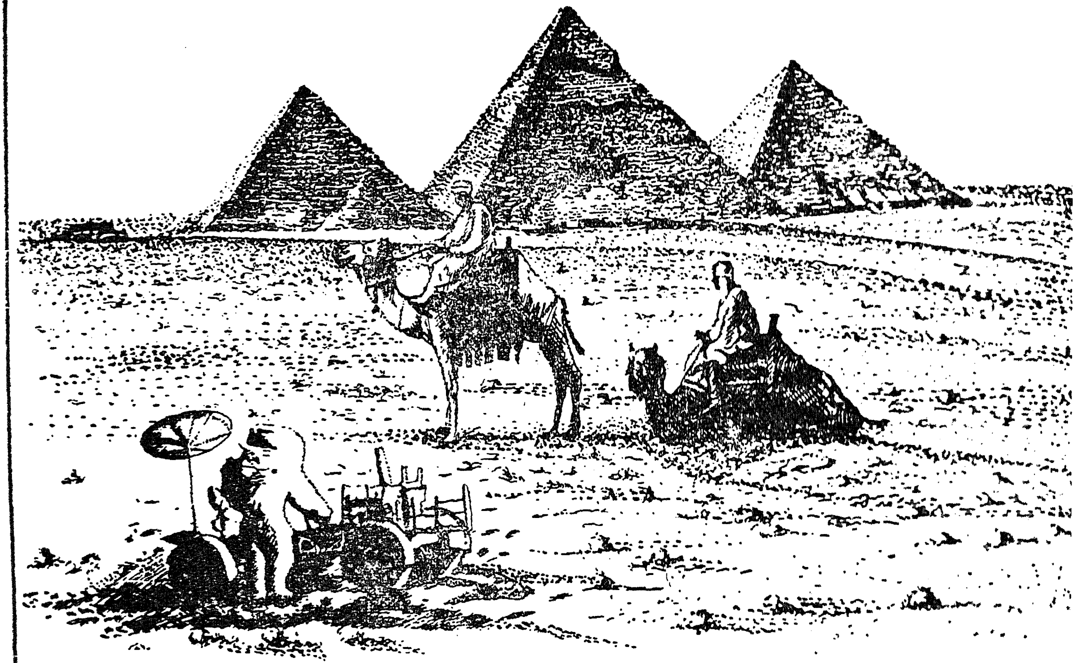
This volume of *Annual Reviews of Physical Chemistry* maintains the high standards of this important series publication. However, one cannot help noticing diversity in the nature and magnitude of coverage in the different review articles. While such a volume should avoid articles listing a catalogue of references, there should still be some uniformity in the presentation of the various reviews. In this volume, some articles are critical reviews of recent developments while others are overview articles; there are still others which are rather simple introductory reviews. In spite of such minor differences, all the reviews in this volume provide good and timely status reports and will undoubtedly be of value to all those engaged in research in the concerned areas.

C. N. R. Rao.

432/77. Published by S. R. S. Sastry, Manager, Current Science Association, Bangalore-560006
and Printed at The Bangalore Press, Bangalore-560018.

Registered with the Registrar of Newspapers for India under 3857/57

Did Space Explorers Visit Ancient Egypt?



Recent theory in Archaeology suggests that visitors from outer space landed in Egypt in prehistoric times.

Evidences such as paintings of spaceships, helmeted creatures, curious coincidences in the matching of the Pyramid's dimensions with distances between the planets and the sun and still inexplicable engineering feats involved in building the Pyramids are salient points for this theory.

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THE BANGALORE PR

THE TWINNING IN CARBONYL PYRAZINE *BIS* (TRIPHENYL ARSINE) RHODIUM (1) PERCHLORATE [Rh (CO) (Pyz) $(Ph_3As)_2$] ClO_4

ANNAPURNA PANDA AND D. S. SAKE GOWDA

Department of Physics, Central College, Bangalore University, Bangalore 560 001

ABSTRACT

The measured cell constants, the observed extinctions of the reflections and the double spots in the Weissenberg photographs, all indicate the 'pseudo-symmetry twinning' in carbonyl pyrazine *bis* (triphenyl arsine) rhodium (1) perchlorate crystals.

INTRODUCTION

THE synthesis of a number of cationic complexes of rhodium(1) has been reported. Carbonyl pyrazine *bis* triphenyl arsine) rhodium(1) perchlorate crystals have been obtained by the reaction of the complex [Rh$(CO)_2$ $(Ph_3As)_3$] ClO_4 with the ligand pyrazine in alcohol (Reddy and Ramesh, 1974[1]).

X-ray study of these crystals were undertaken to collect the preliminary data and the intensity data needed for crystal structure analysis. Because the crystals exhibited twinning, further work on structural analysis was not continued.

During the alignment of the crystals about the crystallographic axes other than the needle axis (*b*-axis), slight deviation from the rotation axes showed considerable distortion of the layer line spots. A number of investigators have attributed that this may be due to twinning in the crystal. In the twinned crystals we have more than a single individual of the same substance. The individuals are so blended that their appearance at first sight is that of a single individual crystal (Tutton, 1922[2]). There are different types of twinning. The pseudo-merohedral symmetry in crystals of para-red (Grainger and McConnel, 1969[3]), the pseudo-symmetry twinning in 5, 5′-bilsoxasole crystals (Cannas, Carta and Marongiu, 1972[4]), the study of the twinned felspars (Ito and Sadanaga, 1952[5]) may be quoted as examples. It is well known that double spots appear in the diffraction pattern due to twin structure. Each reciprocal lattice point, due to one individual of the twin may either coincide or almost coincide with the reciprocal lattice point belonging to the other individual (Cannas, Carta and Marongiu, 1972[4]). The pseudo-symmetry twinning observed in carbonyl pyrazine *bis* (triphenyl arsine) rhodium(1) perchlorate has been reported in this article.

EXPERIMENTAL

The unit cell dimensions of the above crystals were determined using rotation as well as zero layer Weissenberg pictures taken about different crystallographic axes. Copper K_α radiation was used. The crystal lattice is triclinic with cell constats

$a = 18 \cdot 94$ Å, $\alpha = 90°$.
$b = 10 \cdot 31$ Å, $\beta = 70° \, 16'$.
$c = 20 \cdot 54$ Å, $\gamma = 86° \, 59'$.

The number of formula units is two per unit cell. The molecular weight of the compound is 922·88 and its linear absorption coefficient is 66·73 cm^{-1}. The calculated and the measured densities are $d_c = 1 \cdot 57$ gm cm^{-3} and $d_m = 1 \cdot 56$ gm cm^{-3} respectively. The crystal density was measured by floatation technique using carbon tetrachloride and bromobenzene. The measured density and the calculated density are in good agreement indicating two formula units in a cell.

DISCUSSION

Based on cell constants, the crystal lattice is triclinic. But the extinctions observed (*hol*, $l = 2n + 1$) indicate that it belongs to pseudo-monoclinic space group Pc with one of the angles equal to 90°, the other being close to 90°. The cell constants of the crystal, the extinction of the reflections and the double spots in the Weissenberg photographs, all indicate the pseudo-symmetry twinning.

The Weissenberg pictures taken about the needle axis showed no splitting of spots. The zero and the first layer Weissenberg pictures are shown in Fig. 1. The absence of (*hol*) reflections for $l = 2n + 1$ confirms that $c = 20 \cdot 54$ Å which otherwise should be equal to half this value. The zero and the upper layer pictures taken about the other two axes showed splitting of spots. Only the zero and the first layer Weissenberg pictures taken about the *a*-axis are shown in Fig. 2. The Weissenberg pictures taken about the *c*-axis were not good for reproducibility and are not given here.

Regarding the Weissenberg pictures taken about the needle axis, no splitting of spots was observed. This may be due to the fact that the reciprocal lattice point of one individual crystal is coincident with that of the other individual of the twin. With respect to the Weissenberg pictures taken

Shilpi 2 SM 70/73

about the other two axes, the reciprocal lattice of the two individuals of the twinned crystal is non-coincident and gives rise to double spots. It may be concluded that the two individuals of the twinned crystals are formed from independent nuclei during the process of crystallization.

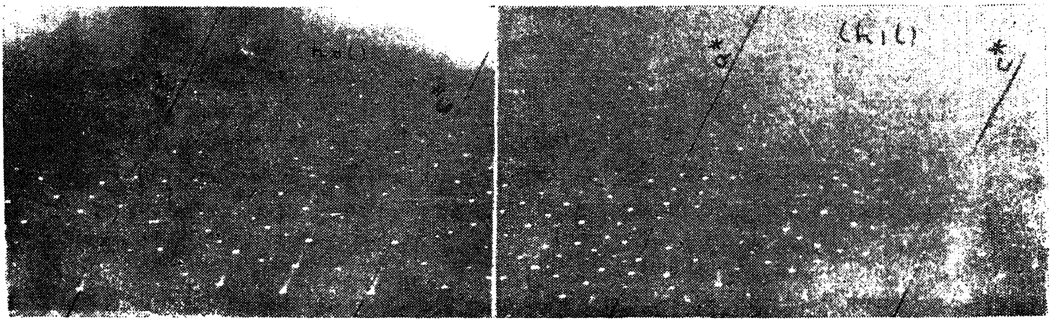

FIG. 1. *hol* and *h1l* Weissenberg pictures no splitting of spots.

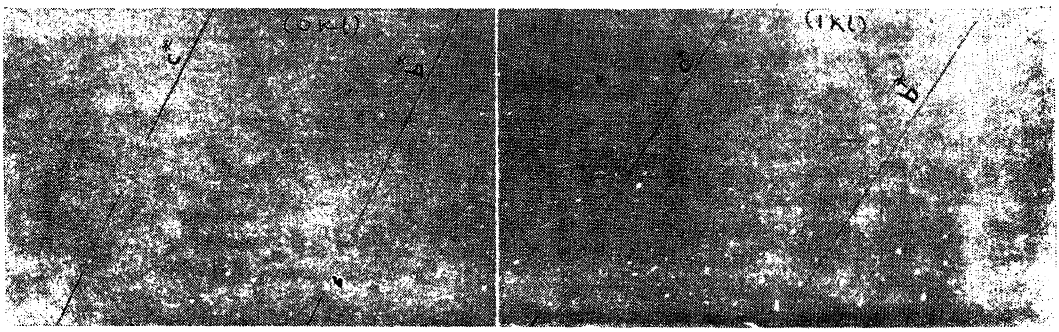

FIG. 2. *okl* and *1kl* Weissenberg pictures showing splitting of spots.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. G. K. N. Reddy, Department of Chemistry, Central College, Bangalore, for supplying the crystals and Prof. K. N. Kuchela, Head of the Department of Physics, Central College, Bangalore, for his encouragement and useful discussions. One of the authors (Annapurna Panda) wish to express her gratitude to the Department of Atomic Energy, Government of India, for providing financial assistance.

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1. Reddy, G. K. N. and Ramesh, B. R., *Journal of Organometallic Chemistry*, 1974, **67**, 443.
2. Tutton, A. E. H., *Crystallography and Practical Crystal Measurement*, Macmillan and Co., Limited, London, 1922, p. 497.
3. Grainger, C. T. and McConnel, J. F., *Acta Cryst.*, 1969, **25 A**, 422.
4. Cannas, M., Carta, G. and Marongiu, *Ibid.*, 1972, **28 A**, 497.
5. Ito, T. and Sadanaga, R., *Ibid.*, 1952, **5**, 441.

STUDIES ON THE TOTAL SYNTHESIS OF 3-DEOXY-7-OXAESTRONE: SYNTHESIS OF 1-DIETHYLAMINO-4-BENZYLOXYBUT-2-YNE AND 1-DIETHYLAMINO-4-BENZYLOXYBUT-2-ENE

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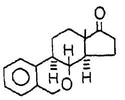
WITH a view to synthesizing the hitherto unknown[1] 3-deoxy-7-oxaestrone (I), the required starting material, 3-benzyloxy-1-propyne[2] (II), was prepared either by treating the sodio-derivative of benzyl alcohol with propargyl bromide or the sodio-derivative of propargyl alcohol with benzyl chloride in refluxing propargyl alcohol. Initially conversion of (II) into the desired Mannich base, 1-diethylamino-4-benzyloxybut-2-yne(III), was attempted adopting strictly the conditions reported by Hughes and Smith[3,4] but the Mannich base (III) was obtained only in low yield (15%). A 46% yield of the desired Mannich base (III) was, however, achieved by treating (II) with a mixture of formalin, water, trioxane, dioxane, diethylamine and glacial acetic acid in the presence of catalytic amount of cuprous chloride at 60–70°. The crude Mannich base (III) on chromatography over Brockman neutral alumina gave a fraction from benzene eluates which on evaporative distillation at 110°/5 mm furnished the analytical sample of 1-diethylamino-4-benzyloxybut-2-yne(III) as a pale yellow liquid in 46% yield. The structure assigned to (III) was based on the following spectral data. The N.M.R. spectrum (CCl_4) indicated signals at δ 1·02 (*t*, 6H, J = 7·5 Hz; $CH_2–\mathit{CH_3}$), 2·48 (*q*, 4H, J = 7·5 Hz, $–\mathit{CH_2}–CH_3$); 3·4 (*t*, 2H, J =2 Hz, $—C{\equiv}C–\mathit{CH_2}–N$) ; 4·13 (*t*, 2H, J = 2 Hz, $–O–CH_2–\mathit{C}{\equiv}C$), 4·53 (*s*, 2H: benzylic CH_2) and 7·22 (*s*, 5H, aromatic). The observed triplets at δ 3·4 and 4·13 with a coupling constant of J=2 Hz, were evidently due to long-range coupling noticed[5] in the case of such acetylenic derivatives.



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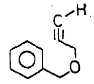

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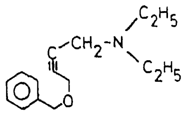
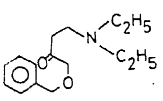


III



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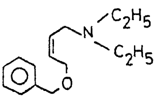
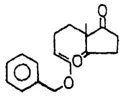


V



VI

Hydration of the triple bond of the Mannich base(III) was next envisaged. Initially, the hydration of (III) was carried out in the conventional manner using mercuric sulphate and sulphuric acid at various temperatures but with little success. Treatment of (III) with a mixture of HgO, trichloroacetic acid and boron trifluoride etherate in methanol according to the procedure reported by Raphael and coworkers[6] also failed to furnish the anticipated ketoamine (IV). In each case the starting base(III) was recovered unchanged as evidenced by mixed TLC and IR comparison with that of the starting material.

The Mannich base(III) was, alternatively, converted to the hitherto unknown 1-diethylamino-4-benzyloxybut-2-ene (V) in almost quantitative yield by partial hydrogenation employing Lindlar's catalyst. The structure (V) was confirmed from its spectral and analytical data. It is hoped that the displacement of diethylamino function by the anion derived from 2-methylcyclopentan-1-3-dione would furnish the anticipated bis-seco-7-oxaestradione(VI). Thus treatment of the Mannich base(V) with 2-methylcyclopentan-1, 3-dione in excess of hot DMSO or NaH in DMSO at room temperature failed to furnish the anticipated secodione(VI). Further work concerning the hydration of the triple bond in the Mannich base (III) under more vigorous conditions is in progress.

EXPERIMENTAL

Preparation of 1-diethylamino-4-benzyloxybut-2-yne (III)

A mixture of 5 g of 3-benzyloxy-1-propyne (prepared from the sodio-derivative of propargyl alcohol and benzyl chloride according to the procedure reported by Guermont[2]), 4·2 ml of formalin (37%), 2 ml of water, 0·4 g of trioxane, 19 ml of dioxane, 4·3 ml of diethylamine, 2·1 ml of glacial acetic acid and 0·1 g of cuprous chloride was heated at 70° for 20 hr The cooled solution was made alkaline with 2 N sodium hydroxide solution and extracted with ether (3 × 50 ml). The ether layer was washed with water and extracted with 3 N HCl (3 × 30 ml). The resulting acidic solution was washed with ether and then made alkaline with 2 N NaOH solution. The oily liquid thus separated was extracted with ether (3 × 50 ml) and the ethereal layer was thoroughly washed with water (4 × 20 ml). The ether layer, dried over Na_2SO_4 was evaporated. The crude Mannich base (6·76 g) was chromatographed over Brockman neutral alumina (180 g). The fraction obtained from the benzene eluates on evaporative distillation at 115°/5 mm furnished the analytical sample of (III) (3·94 g) in 46% yield; λ_{max} (95% EtOH) 221 (ϵ, 2, 021) and 242 mm, *sh* (ϵ, 924) ; IR ($CHCl_3$) bands at 1600, 1595 (aromatic C=C), 1105, 1090 and 1070 cm^{-1} (C–O–C, antisymmet. stretch) ; mass spectrum indicated peaks at m/e 231 (5% ; M^+ ion), m/e 216 (33%), m/e 92 (10%), m/e 91 (100%) and m/e 77 (12%). (Found : C, 77·70, H, 9·09, N, 5·91%. $C_{15}H_{21}ON$ requires C, 77·87, H, 9·09, N, 6·05%).

Preparation of 1-diethylamino-4-benzyloxybut-2-ene(V)

A solution of 0·29 g of 1-diethylamino-4-benzyloxybut-2-yne(III) in 10 ml of pure ethyl acetate (distilled from Raney Nickel and dried over anhydrous Na_2SO_4) was stirred with 0·03 g of Lindlar catalyst (Fluka, Buchs, S.G.) in an atmosphere of hydrogen until the absorption of hydrogen ceased. After the usual work-up, the resulting yellow liquid on distillation at 140°/5 mm furnished the analytical sample of (V) (0·28 g). TLC showed a single spot (benzene : ethyl acetate : tert. butanol as 20 : 4 : 1) ; λ_{max} (95% EtOH) 221 (ϵ 2,183) and 242 nm (*sh*) (ϵ 904) ; IR (Smear) bands at 1600, 1580, 1490 (aromatic and olefinic C=C), 1090 and 1070 (C–O–C antisymmet. stretch); NMR (CCl_4) signals at δ 0·93 (t, 6H, J=7 Hz, CH_2–CH_3), 2 43 (q, 4H; J=7 Hz, CH_2–CH_3), 2·98 (d, 2H, J=4 Hz, allylic coupling CH_2–N), 4·0 (d, 2H, J=4 Hz; allylic coupling, O–CH_2–CH=CH–) 4·4 (s, CH_2, benzylic), 5·6 (m, olefinic protons); mass peaks at m/e 233 (0·2% M+), m/e 91 (100%) and m/e 77 (7%) (Found: C, 77·20, H, 9·6 and N, 6·0%. $C_{15}H_{23}ON$ requires C, 77·25, H, 9·8, N, 6 03%).

ACKNOWLEDGEMENT

One of us (A. P. C.) is thankful to the Director of this Institute for financial assistance.

1. (a) Ramadas, S. R. and Radhakrishnan, J., *J. Sci. Ind. Res.*, 1972, **31**, 145.
   (b) — and Chaudhuri, A. P., *Ibid.*, 1975, **34**, 563.
2. Guermont, J. P., *Mem. Serva. Chim.* (*Paris*), 1955, **40**, 167.
3. Smith, H., Hughes, G. A. and Walk, C. R., *Experientia*, 1964, **20**, 418.
4. Hughes, G. A. and Smith, H., *Proc. Chem. Soc.*, 1960, **74**.
5. *Applications of NMR Spectroscopy in Organic Chemistry*, Ed. by Jackman, L. M. and Sternhell, S., Pergamon Press (London), 2nd Ed., 1969, p. 328.
6. Parker, W., Ramage, R. and Raphael, R. A., *J. Chem. Soc.*, 1962, p. 1558.

EFFECTS OF DENERVATION ON THE CALCIUM SENSITIVITY OF SUCCINATE DEHYDROGENASE IN THE AMPHIBIAN GASTROCNEMIUS MUSCLE

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ABSTRACT

The effects of calcium and EDTA were studied on SDH activity levels in the denervated and contralateral control muscles. Calcium and EDTA were found to alter the V_{max} value of the enzyme, the former increasing while the latter decreasing but with no effect on the Km. Though calcium was found to modulate positively the SDH activity in denervation atrophy, it is possible that the incapacity in *in vivo* may be due to a shift in metabolic involvement of Ca^{2+} towards the membrane properties and function.

INTRODUCTION

THE denervated and dystrophic muscles exhibited an increase in the Ca^{2} content[1-4]. This calcium accumulation was found to appear early in the progression of the muscle disease[5]. The same muscles were found to be defective in oxidative phosphorylation[3]. The importance of calcium in muscle physiology has been recognized since the classical studies of Ringer[6] and Lehninger[7] indicated that the intracellular regulation of calcium by mitochondria take primacy over oxidative phosphorylation. Since the denervated muscle has elevated calcium levels and low oxidative enzyme activity, it is worthwhile to see how the calcium and oxidative enzyme activity are correlated.

MATERIAL AND METHODS

Rana hexadactyla (60–80 g) were used in the present investigation. Muscle atrophy was induced in the frogs by sciatic nerve section. The thigh of one leg was opened and a length of 1–1·5 cm of sciatic nerve was removed under aseptic conditions. The contralateral muscle of the other leg was considered as control. The frogs were maintained in aquarium tanks being daily fed with cockroaches.

After 30 days of operation, the frogs were double pithed and the gastrocnemius muscle of both legs was isolated and immediately chilled. The tissues were homogenised in 0·25 M sucrose solution to 10% (W/V) and centrifuged at 2500 rpm for 15 min to remove the cell debris. The supernatants were used for the assay of enzyme activity. The succinate dehydrogenase (SDH: EC 1.3.99.1) activity was assayed by the method of Nachlas *et al.*[8] and the enzyme activity was expressed as μMoles of formazan formed/mg protein/hr. Protein levels were determined by the method of Lowry *et al.*[9] 2 mM of calcium chloride and 3 mM of EDTA (Ethylene diamine tetra acetic acid) were selected after preliminary standardization.

RESULTS AND DISCUSSION

The activity levels of SDH were 0·112 and 0·173 μMoles in the denervated and contralateral control muscles respectively, showing a drop of 35% in muscle as a consequence of denervation atrophy. Similar decrease in SDH activity of the muscle after denervation has been reported earlier[10]. The activity level of SDH in both the denervated and contralateral control muscles was found to be increased by calcium. It increases the maximal velocity from 0·143 to 0·34 μMoles in the control muscle and from 0·119 to 0·330 μMoles in the denervated muscle. The Km values in the absence and presence of calcium were 0·38 and 0·37 mM in the control muscle and the corresponding values in the denervated muscle were 0·58 and 0·59 mM. This type of activation where only V_{max} was effected but not the Km indicates that the activation pattern is of non-competitive type (Figs. 1*a* and 1 *b*). The non-competitive type of activation by calcium suggests that it reacts with the enzyme at a site other than the active site bringing about conformational changes and making the enzyme active sites catalytically effective. To confirm the role of calcium, the effect of EDTA was studied.

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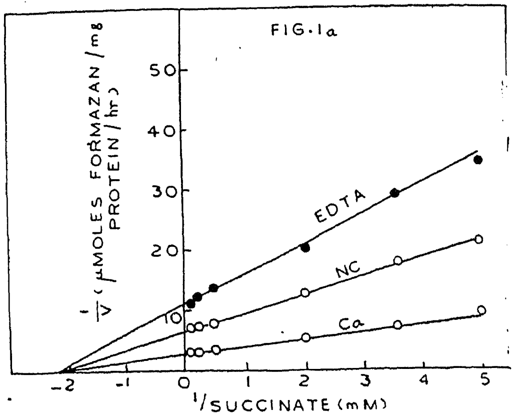

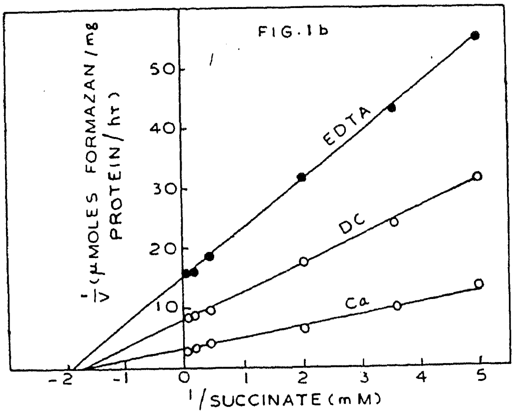

FIGS. 1*a* and 1*b*. Double reciprocal plots showing non-competitive inhibition of SDH of the control (Fig. 1*a*) and denervated (Fig. 1*b*) muscles by EDTA and non-competitive activation of the enzyme by calcium (Ca).

EDTA was found to elicit an inhibition on the activity of the enzyme from both the control and denervated muscles. The V_{max} values for the control muscle enzyme were 0·143 and 0·094 μMoles in the absence and presence of EDTA respectively. The corresponding values for the denervated muscle enzyme were 0·119 and 0·065 μMoles. The Km values were found to be 0·38 and 0·39 mM in the control muscle in the absence and presence of EDTA respectively. In the denervated muscle the corresponding values were found to be 0·58 and 0·55 mM. The inhibition by EDTA may be of non-competitive type decreasing the maximal velocity with relatively no effect on the enzyme-substrate affinity (Figs. 1 *a* and 1 *b*).

The activation of SDH by calcium in both the normal and denervated muscles can be attributed to its role in regulating the flow of metabolites across the mitochondrial membrane[11]. It is likely that the calcium may facilitate the entry of succinate into the mitochondria. The inhibitory action of EDTA may be due to the binding of calcium ions which are shown to positively modulate SDH activity of the normal and denervated muscles.

The per cent calcium activation of denervated muscle enzyme was 177% while the contralateral control muscle enzyme was only 137% indicating that the calcium sensitivity of muscle enzyme increases on denervation atrophy. The per cent inhibition by EDTA was 34 and 45 in the control and denervated muscles respectively. Thus the denervated muscle enzyme was found to be inhibited more by EDTA when compared to that of the control muscle. It was reported earlier that the denervated frog muscle becomes markedly sensitive to extracellular calcium[12]. However the elevated free calcium levels reported in the denervated muscle[2] should have kept up the denervated muscle SDH activity. Probably, the elevated Ca^{2} content in the denervated muscle might have shifted its involvement in the spontaneous myofibrillar contractions which were found to predominate in the denervated muscle since the denervated muscle was found to need higher amounts of calcium to maintain the muscle membrane properties than the control muscle[13]. Similar accumulation of calcium in sarcoplasm of the muscle was observed during repeated contractile activity resulting in the onset of fatigue[15]. In spite of the high calcium contents, the fatigued muscle was found to have low SDH activity when compared to the normal muscle[10].

Godwin *et al.*[14] showed that the earliest abnormality during the onset of selenium deficiency may be with respect to an ion, the redistribution of which would be expected to cause profound changes in muscle integrity. Hence, it is likely that the shift in the functional involvement of calcium in the muscle for the metabolic enzyme to membrane potentials may be responsible for the lower oxidative enzyme activity or the degenerative processes in general set in the atrophic muscle.

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1. Shimizu, S. and Kuriaki, K., *Am. J. Physiol.*, 1960, **198**, 943.
2. Issacson, A. and Sandow, A., *J. Pharmac. exp. Ther.*, 1967, **1955**, 376.
3. Wrogemann, K., Jacobson, B. E. and Blanchaer, M. C., *Can. J. Biochem.*, 1973, **52**, 500.
4. Godwin, K. O., Jenny Edwardly and Fuss, C. N., *Aust. J. Biol. Sci.*, 1975, **28**, 457.
5. —, Kuchel, R. E. and Fuss, C. N., *Ibid.*, 1974, **27**, 633.
6. Ringer, S., *J. Physiol.* (Lond.), 1883, **4**, 29.
7. Lehninger, A. L., *Biochem. J.*, 1970, **119**, 129.
8. Nachlas, M. M., Margulius, S. P. and Seligman, A. M., *J. Biol. Chem.*, 1960; **235**, 2739.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
10. Muralikrishna Das, P. and Swami, K. S., *Enzymologia*, 1972, **42**, 235.
11. Hainaut, K. and Desmedt, J. E., *Nature*, 1974, **252**, 407.
12. Albert Kirby, C., Lindley, B. D. and John Picken, R., *Am. J. Physiol.*, 1973, **225**, 166.
13. Thesleff, S. and Ward, M. R., *J. Physiol.*, 1975, **244**, 313.
14. Godwin, K. O., *Aust. J. Exp. Agric. Anim. Husb.*, 1972, **12**, 473.
15. Web, A. and Bremel, R. D., In: *Contractility of Muscle Cells and Related Processes* (Ed. Richard J. Podolsky), Prentice-Hall, Inc., 1971.
16. Kumudavalli, I., Swami, K. S. and Surendranath Reddy, Y., *Ind. J. exp. Biol.*, 1967, **5**, 178.

PLANT GROWTH REGULATORS AND SEX EXPRESSION IN FLOWER BUDS OF *MOMORDICA CHARANTIA IN VITRO*

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ABSTRACT

A study on the effect of plant growth regulators on the sex expression of *Momordica charantia in vitro* revealed that α naphthalene acetic acid, kinetin and ethrel induced femaleness by increasing the size of pistillage buds while gibberellic acid and abscisic acid induced maleness by increasing the size of staminate buds. α naphthalene acetic acid and gibberellic acid were more effective than other plant growth regulators in causing femaleness and maleness, respectively. Morphactin caused maximum male sterility.

INTRODUCTION

THE *in vitro* culture of plant tissues has been studied by Gautheret[5,6] and his coworkers from 1940. The effect of plant growth regulators on sex expression in flower buds *in vitro* is very interesting and recently Galun *et al.*[3,4] studying the effect of growth regulators like IAA and GA$_3$ in flower buds of *Cucumis sativus* reported that IAA promote the development of ovary and this effect is antagonised by GA$_3$. Their interesting findings gave impetus to undertake this problem. One of the advantages of using this type of study is that nutrients and plant growth regulators of known concentration may be substituted for those normally available in the intact plants. Under these conditions, it is then possible to single out any one factor and determine its influence on sex expression under controlled conditions. The present experimental study includes not only α naphthalene acetic acid and gibberellic acid but also other plant growth regulators like kinetin, abscisic acid, ethrel and morphactin.

MATERIALS AND METHODS

The buds (2·5 cm in length) of *Momordica charantia* grown under normal conditions were dipped in distilled water just after picking followed by 1·5% sodium hypochlorite solution and finally washed with distilled water three or four times. After autoclaving the 2% agar medium appropriate amounts of α naphthalene acetic acid (α NAA), gibberellic acid (GA$_3$), kinetin (KN), abscisic acid (ABA), ethrel (Eth) and morphactin (CFl) were weighed and mixed in culture medium. The culture medium was the same as used by Henderson *et al.*[7], with slight modification, *i.e.*, KN and IAA were not used as the constituents of culture medium. Each plant growth regulator was prepared at 5 ppm concentration. Plant regulator free basic medium was taken as control. The agar nutrient medium either with or without plant growth regulators was poured in deep sterile petridishes to a 5·00 mm height and 3·00 mm of lower ends of flower buds were embedded in the culture medium. The flower buds were incubated at 22 ± 2° C. Eight replicate cultures were used for each treatment. The reduction in opening of staminate flowers from staminate buds was taken as a criterion for pollen sterility. This was further confirmed by using the safranin stain, given to the pollen.

TABLE I

Effect of plant growth regulators on the size of flower buds and % of open staminate flowers of Momordica charantia in vitro

Sl. No.	Treatments	Size of flower in cm				% of open staminate flowers
		Staminate flower		Pistillate Flower		
		Length	Diameter	Length	Diameter	
1.	Contro	3·8±·12	1·8±·10	3·6±·16	1·9±·14	80
2.	α NAA	3·0±·14	1·6±·10	5·2±·18	2·7±·16	64
3.	GA_3	8·1±·21	2·0±·12	3·8±·18	2·0±·14	96
4.	KN	4·0±·18	1·9±·12	4·3±·20	2·6±·16	80
5.	ABA	4·6±·16	2·4±·14	2·7±·14	1·4±·10	88
6.	Eth	4·2±·16	2·2±·14	5·3±·20	2·6±·16	64
7.	CFl	2·8±·12	1·2±·10	2·6±·10	1·3±·12	16

Observations were made on flower size in terms of length and diameter measured at 3 points-base, middle and apex. The cultures were maintained for 15 days. The data given in the table are expressed as average size ± standard error of flower buds treated with various plant regulators. The '*t*' test was used as a measure of statistical significance.

RESULTS AND DISCUSSION

The results have been shown in Table I. *In vitro* experiments reveal that all the plant regulators influenced the size of both kinds of flower buds. α naphthalene acetic acid increased the size (length and diameter) of pistillate flower buds and decreased the length of staminate type. Percentage of open staminate flowers was reduced from 80 to 64% and show pollen sterility. Decrease in length of staminate flower bud and increase in size (length and diameter) of pistillate type show the female tendency by α NAA. This tendency has also been reported by Choudhury and Phatak[2] in intact plants of *Cucumis sativus* and Satyanarayana and Rangaswami[12] in ribbed gourd.

Gibberellic acid, a well-known plant growth regulator for cell elongation also caused elongation in flower buds but this elongation was confirmed to only staminate buds. It had no significant effect on the size of pistillate flower which showed that GA_3 without influencing pistillate flower, induced maleness. This tendency increased with an increase in percentage of open flowers which showed a decrease in pollen sterility. Maleness caused by GA_3 has also been shown by Atsmon *et al.*[1] in cucumber and by Randhawa and Singh[10] in muskmelon. Kinetin caused an increase in the size of only pistillate flowers which showed the tendency of KN towards femaleness. Feminization due to KN treatment has also been observed by Negi and Olmo[9] and Moore[8] in *Vitis vinifera.*

Abscisic acid reduced the size of pistillate flower but increased the size of staminate flowers. Abscisic acid (by decreasing the size of pistillate flower and increasing the size of staminate flower) enhanced maleness by depressing the femaleness. Like GA_3, ABA also reduced the pollen sterility by increasing the percentage of open staminate flowers. Ethrel had no effect upon staminate flowers but increased the length and diameter of pistillate flower buds. Besides feminization ethrel showed male sterility also by decreasing the percentage of open staminate flowers.

Morphactin, a growth retardant decreased the size of flower buds of both staminate and pistillate types. Morphactin greatly enhanced pollen sterility by decreasing the percentage of open staminate flowers. Sankhla and Sankhla[11] and Uma *et al.*[13], reported abortion of stamens in *Nicotiana peniculata* and *Cannabis sativa,* respectively.

Among all the treatments, maximum reduction in size of the staminate and pistillate flower buds was noted in CFl treatment. Maximum length was noted in GA_3 treated staminate and Eth treated pistillate flower buds. Maximum diameter for staminate and pistillate flower buds was noted in treatments with ABA and α NAA, respectively. Gibberellic acid caused maximum increase and

CFI caused maximum decrease in percentage of open staminate flowers.

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1. Atsmon. D., Lang, A. and Light, E. N., *Plant Physiol.*, 1968, 43, 803.
2. Choudhury. B. and Phatak, S. C., *Indian Jour. Hort.*, 1959, 12, 143.
3. Galun, E., Jung, Y. and Lang, A., *Nature*, 1962, 194, 596.
4. —. — and —, *Devl. Biol.*, 1963; 6, 370.
5. Gautheret, R. J., *La Culture des tissue Gallimard. Paris*, 1945.
6. —, *Plant Tissue Culture*, Sixth Growth Symposium, 1947, 21.
7. Henderson, J. H. M., Durrell, M. F. and Bonner, J., *Am. J. Bot.*, 1952, 39, 467.
8. Moore, J. N., *Jour. Amer. Hort. Sci.*, 1970, 96, 387.
9. Negi, S. S. and Olmo, H. P., *Science*, 1966; 152, 1624.
10. Randhawa, K. S. and Singh, K., *Plant Science*, 1969, 1, 69.
11. Sankhla, N. and Sankhla, D., *56th Proc. Ind. Sci. Cong.*, Pt. III, 1969, p. 415.
12. Satyanarayana, G. and Rangaswami, G., *Curr. Sci.*, 1959, 28, 376.
13. Uma, M. C., Mehta, Usha, Illyas Mohammed, Jaiswal, V. S. and Mohan Ram, H. Y., *Proc. Sym. Biol. Land Plants*, 1972, 33.

SIXTY YEARS OF COCONUT RESEARCH AND DEVELOPMENT IN INDIA

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THE Indian Society for Plantation Crops and the Indian Council of Agricultural Research celebrated the Diamond Jubilee Year of Coconut Research in India from December 27, 1976 to January 8, 1977. Coconut research was first begun in India (and also in the world) in 1916 at Kasaragod, Nileshwar, and Pilicode under the old Madras Presidency. Today, Kasaragod is the headquarters of the Central Plantation Crops Research Institute, Indian Council of Agricultural Research. It is the main Institute in India researching on coconut. The Coconut Research Station at Nileshwar is under the Kerala Agricultural University and the Pilicode Station functions under the Kerala Agriculture Department.

The most notable achievements of six decades of coconut research has been the production of hybrids between Tall and Dwarf forms of coconuts and discovery that they are high yielding, early bearing and semi-tall as compared to local forms. Further, they bear better even when infected by the dreaded root (wilt) disease. A commemorative stamp brought out by the Posts and Telegraphs Department depicted a semi-tall, early bearing hybrid palm of coconut.

The coconut palm is best known as an oil yielding plant. Coconut oil ranks sixth in the world today in terms of production and fourth in terms of international trade among edible oils. India ranks third in the world after the Philippines and Indonesia in coconut production. In India, coconut ranks about the fourth in production among the vegetable oils.

Till about four decades ago, coconut oil was the leading edible vegetable oil of international trade. It was also among the cheapest oils. Today, it has become one of the most expensive vegetable oils. It is in this background that the present Symposium was organized. Incidentally, this was the first time that an international symposium was organized on this important and most useful plant.

The Symposium was attended by 320 scientits and delegates from all over India and most of the coconut growing countries of the world. It was inaugurated by Dr. M. S. Swaminathan, FNA, FRS (Director General, Indian Council of Agricultural Research and Secretary to the Government of India, Department of Agricultural Research and Education), with a keynote address 'Coconut research—the next phase'. He identified the major R & D problems in coconut as breaking the yield barrier which has remained at best stagnant with 30-40 nuts annually. He also called for finding out a solution to the root (wilt) disease of Kerala State which is responsible for an annual loss of Rs. 300 million.

The Symposium was organized in 9 sessions in which 80 papers were presented, 46 from India and 34 from abroad. These sessions consisted of Genetics and Plant Breeding (10 papers), Agronomy and Soi Chemistry (10 papers), Biochemistry and Physiology (9 papers), Technology (7 papers), Basic Studies (7 papers), Diseases (5 papers), Diseases of Uncertain Aetiology (10 papers), Pests (7 papers), and Development Programmes in India and Other Parts of the Country (16 papers).

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* Chairman, Programme Committee, ISOCRAD.

The main emphasis of the papers, presented in the Genetics and Plant Breeding Session, related to the development of methods for identifying high yielding and prepotent palms based on characters of coconut seedlings in the nursery; and, the characteristics of dwarf forms of coconut and the need for intensifying studies on them for effectively utilizing them in varietal improvement programmes. The papers in Agronomy and Soil Chemistry emphasised the need to increase the productivity of coconut gardens by intercropping them with other crops as cacao or by mixed farming with fodder grass and legumes, raising milch cattle, and recycling cattle waste; and manurial and water requirement studies on coconut. The papers in Biochemistry and Physiology discussed biochemical changes during maturation and germination of coconut, vascular physiology of coconut palm, varietal differences in nutrtive values of copra and glucose content of coconut water, and description of a method for more efficient fermentation of toddy. Perhaps the widest field was covered in the session on Technology in which the papers dealt with the methods of wet milling fresh coconut kernel, the 'solvol' process developed by an Indian firm for an integrated and total utilization of all parts of the coconut fruit from husk to coconut water, utilization of coconut husk as a cheap and durable roofing material, or for making utility items as coir, matresses, brushes, etc., a review of the microbiological studies involved in retting of coconut husk, and production of activated carbons from fibre pith and coconut shell. In the session on Basic Studies, two papers elicited considerable interest. One presented data on floral biology and fruiting of coconut trees raised through embryo culture and the second reviewed the progress attained on vegetative propagation of coconut palm. Vegetative propagation in the coconut has assumed more relevance because of the recent success obtained in raising oil palm from tissue culture. The method offers great advantages for the improvement of coconut palm because of its long juvenile phase and its obligate seed propagation. Two other papers dealt with association of foliar asymmetry with latitude and origin of coconut.

The highlights of the Symposium were the two Sessions on Diseases and Diseases of Uncertain Aetiology. Understandabiy, because the coconut palm is affected by a number of diseases, several of uncertain aetiology. Several papers presented in the Symposium brought out that co-operative and systematic researches that are in progress in some of the centres are beginning to produce positive results and the scientists are "closing in" (to quote from Dr. Swaminathan) on determining the aetiology of several of them. Theparticipants hoped that similar efforts would be put forth on the root (wilt) disease of Kerala State also. On root (wilt), Maramorosch (Rutgers, USA) proposed that even though its aetiology was uncertain, its spread could be prevented by manipulating interactions between disease agents, plant hosts, and insect vectors. There are also two other diseases in India whose aetiology has not been properly determined, namely, Thattipaka disease of Andhra Pradesh and Thanjavur wilt of Tamil Nadu. Then, there are other diseases elsewhere in the world like the anabe, bud rot, red ring, hart rot, lethal yellowing and the kainkope disease which are responsible for several losses and are only little understood.

The session on Pests generally emphasised the need for adopting improved methods to control them. However, the serious damage being caused by the rhinoceros beetle (*Oryctes rhinoceros*) and leaf eating caterpillar (*Nephantis serinopa*) in many coconut growing countries of the world were pointed out repeatedly by several workers for initiating co-ordinated research efforts on them. The largest number of papers were presented in the Session on Development Programmes. More than anything else, it exposed the inadequacy of the present extension services in transferring the results of research available in research institutes and agricultural farms to the farmers. In this context, the organization of the coconut industry boards of Sri Lanka and Jamaica was commended for adoption by other coconut growing countries.

In the Plenary Session. the delegates were unanimous that this first international symposium on coconut research was highly successful in bringing together coconut scientists from all over the world. It decided, therefore, to form a permanent secretariat with its headquarters at Kasaragod, Kerala State, with representatives from important coconut growing countries of the world to maintain and strengthen the existing contacts and to organize similar symposia in future.

Another notable feature of the Celebrations was a two-day Travelling Seminar following the Symposium, with about 10 scientists each from the CPCRI, other laboratories in India and from abroad. The seminar discussed the diseases of uncertain aetiology in India. Though several decisions were taken, its main fall out appeared to be the consensus that the work now in progress at Kayengulam was basically on right lines, but reseaches particularly on viral and soil microbiological aspects required to be improved and strengthened.

The proceedings of the Symposium will be published by the end 1977 by Wiley Eastern Limited, New Delhi.

DR. M. S. SWAMINATHAN, F.R.S., ELECTED FOREIGN ASSOCIATE OF THE NATIONAL ACADEMY OF SCIENCES, UNITED STATES

Dr. M. S. Swaminathan, Director General, Indian Council of Agricultural Research (ICAR) has been elected Foreign Associate of the National Academy of Sciences of the United States for his outstanding contributions to applied genetics and biology. Dr. Swaminathan, who had earlier been elected a Fellow of the Royal Society of London (FRS), is the first Indian Scientist who has been honoured so far, both by the Royal Society of London and the US National Adademy of Sciences. Dr. Swaminathan is the third Indian Scientist to get this honour from the US National Academy of Sciences. Dr. Swaminathan is a Fellow of the Indian National Science Academy, and the Indian Academy of Sciences and an Honorary Fellow of the National Academy of Sciences of India, Allahabad.

Dr. Swaminathan has been the recipient of scientific awards, including the Shanti Swaroop nagar Award, Birbal Sahni Medal of the Botanical Society, Mendel Centenary Award Czechoslovak Academy of Sciences and the Jubilee Commemoration Medal of the Indian N Science Academy. In 1971, he was awarde Ramon Magasaysay Award for Community ship for his contribution as "Scientist, Educa students and farmers and Administrator resp for generating a new confidence in India's A tural capabilities". The membership of the N Academy of Sciences of the United States is purely on the quality of scientific work and its to human welfare.

AWARD OF RESEARCH DEGREES

Osmania University, Hyderabad, has awarded the Ph.D. degree in Physics to Shri V. Govinda Reddy, Ph.D. degree in Zoology to Shri Shah Mohd. Shahnoor.

Berhampur University, Berhampur (Orissa), has awarded the Ph.D. degree in Zoology to Shri G. K. Panigrahi.

Karnatak University, Dharwar, has awarded the Ph.D. degree in Mathematics to Shri Shetty Dharanappa Jinnappa, Ph.D. degree in Zoology to Shri Raichuri Nazeer Ahmad.

M.S. University of Baroda, Baroda, has awarded the Ph.D. degree in Botany to Smt. Aparna Dasgupta, Ph.D. degree in Biochemistry to Shri Deep Bhandari and Kumari Saramma Kuriakose.

University of Cochin, Cochin (Kerala), has the Ph.D. degree in Marine Sciences to Madhupratap.

Sri Venkateswara University, Tirupati, has the Ph.D. degree in Chemistry to Shri S. C. Rajan.

Kakatiya University, Warangal, has award Ph.D. Degree in Electrical Engineering to Subramanyam, Ph.D. degree in Chemistry A. C. Hiremath, Ph.D. degree in Chemistry Purna Chandra Dalai.

Utkal University, Bhubaneswar, has award Ph.D. degree in Chemistry to Shri B. Tulas Pravati Misra, Ph.D. degree in Geology to Sh Rao, Ph.D. degree in Chemistry to Shri B. C and Ph.D. degree in Zoology to Shri N. K. Mah

LETTERS TO THE EDITOR

STUDIES ON POLY(ACRYLONITRILE-CO-VINYLPYRROLIDONE)

It has been reported that polyacrylonitrile fibre is difficult to dye but copolymers of acrylonitrile with small amounts of other vinyl monomers are easily dyed[1]. Eventhough a number of patents have been filed[2-8] it has been found that the reactivity ratios of acrylonitrile with vinyl pyrrolidone and subsequent property changes that are imparted to polyacrylonitrile fibres have not been reported in open literature. This paper deals with the preparation of the copolymer and a novel method has been used to determine the reactivity ratios. The affinities of various dyes towards the homopolymer and the copolymer have been compared.

Both monomers, acrylonitrile (AN) and vinyl pyrrolidone (VP) were purified by double distillation under a reduced nitrogen atmosphere. Copolymerization runs for different monomer feed ratios were carried in dimethyl formamide using α, α' azobisisobutyronitrile as initiator at 56° C under nitrogen. In every polymerization run, the conversion of monomer to polymer was restricted to 10% in order to avoid heterogeneity in composition and possible chain branching. The copolymer was purified by dissolving in DMSO and precipitating with methanol. The results of polymerization are summarized in Table I.

Determination of reactivity ratios

The reactivity ratios were determined by using the differential equation[9], of the copolymer composition

$$\frac{d[M_1]}{d[M_2]} = \frac{[M_1]}{[M_2]}\,\frac{r_1[M_1]+[M_2]}{r_2[M_2]+[M_1]} \qquad (1)$$

At low conversion $d[M_1]/d[M_2]$ becomes m_1/m_2 (the monomer mole ratios found in the copolymer) and M_1 stands for acrylonitrile. Substituting F for $[M_1]/[M_2]$ mole fraction ratios in the monomer feed and f_1 for m_1/m_2 in equation (1) and rearranging the results

$$\frac{F}{f}(f-1) = r_1\frac{F^2}{f} - r_2 \qquad (2)$$

which can be further rearranged to

$$\frac{f-1}{F} = \frac{-r_2 f}{F^2} + r_1 \qquad (3)$$

From equation (2) a plot of $F/f(f-1)$ *versus* F^2/f gives a straight line whose slope is r_1 and intercept r_2. From equation (3) a plot of $(f-1)/F$ *versus* f/F^2 gives the slope r_2 and the intercept r_1. The advantage of having both forms, of the equation is that in some cases one gives a better straight line than the other. In the present case both these methods were adopted which gave identical values of r_1 and r_2 (Fig. 1).

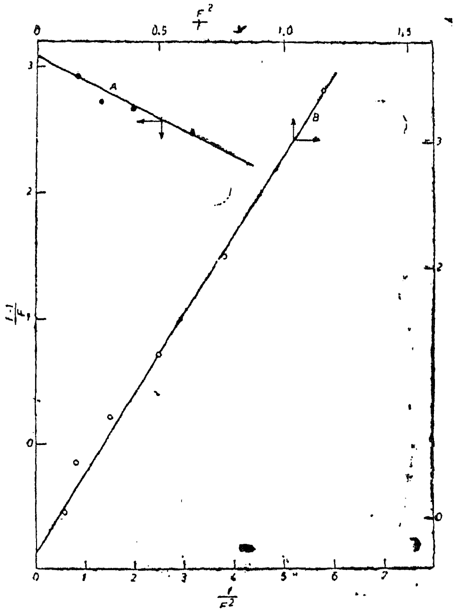

FIG. 1

The monomer reactivity ratios were determined by the analysis of infra-red spectra. Since both the monomers contain nitrogen, elemental analysis cannot determine the composition of copolymers. Thin films of the samples were cast on glass plates using 1% copolymer in dimethyl sulphoxide. On evaporation of the solvent under vacuum at 55° C, transparent films were obtained. For the standard 1:1 weight ratios of individual homopolymers were intimately mixed and the solution in DMSO cast on glass plate. The infra-red spectra were taken using Beckmann IR 20 double beam grating spectrometer. From the ratios of the corresponding peak areas 2210 cm⁻¹ for $C{\equiv}N$ and 1650 cm^{-1} for $C{=}0$, the compositions were calculated as shown in Table I. The reactivity ratios were obtained from the plots of F^2/f *vs.* $(F-F)/f$ and $(f-1)/F$ *vs.* f/F^2, to be $r_1 = 3{\cdot}1$; $r_2 = 0{\cdot}23$.

An attempt has been made to compare dyeability of polyacylonitrile homopolymer and the polyacrylonitrile-co-vinylpyrrolidone of 1:1 composition. In the copolymer the presence of carbonyl group and its neighbouring position to tertiary nitrogen should increase the dyeability of both acid and basic dyes. 1% solution of Rhodamine T and acid orange II were used. The material to liquor ratio was 1: 40.

TABLE I

Copolymerization of acrylonotrile and vinylpyrrolidone at 56° C initiated by AIBN

AN mole % feed	AN in copolymer	*f* obs.	*f* calcd.	F^2/f	$(F - F)/f$
0·2852	0·5398	1·170	1·360	0·137	0·0584
0·4076	0·7535	3·018	2·303	0·156	0·4603
0·5171	0·7852	3·650	3·650	0·313	0·7770
0·6162	0·8404	5·268	5·268	0·489	1·3012
0·7060	0·8838	7·598	7·598	0·763	2·0920
0·7893	0·9240	11·947	11·847	1·174	3·4300

The dyeing was carried out at 75° C for 1 hour. The residual dye solution was estimated by visible spectrum using *uv* vis specord. The percentage of the dye uptake is given in Table II. The dyeability was increased with respect to both the acid and the basic dyes.

TABLE II

Dye uptake by polyacrylonitrile and poly(acrylonitrile-Co-vinylpyrrolidone)

Sample	Rhodamine T dye uptake %	Acid orange II Dye uptake %
PAN film	16·40	15·00
Copolymer film	20·50	28·50

The authors are very grateful to General Aniline and Film Corporation, USA, for supplying vinylpyrrolidone as gift sample.

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1. Bruno Vollmert, *Polymer Chemistry*, Springer-Verlag, 1973.
2. U.S. Patent 2,643,9990 (Ham, G. E. to Chemstrand Corp.); *Chemical Week*, Sept. 8, 1956, p. 85.
3. Brit. Patent 722,790 (Celanese Corp. of America).
4. Australian Patent Application 23495/56 (Dow Chemical Co.) through *Chemical Week*, August 31, 1957, p. 89.
5. Brit. Patent 722,523 (Ham, G. E. and Craig, A. B. to Chemstrand Corp.).
6. U.S. Patent 2,768,148 (Schildknecht, C. E. and Wallace, M. L. to Cellulose Corp. of America).
7. U.S. Patent 2,713,573 (Schildknecht, C. E. and Wallace, M. L. to Cellulose Corp. of America).
8. German Patent 922,378 (Reppe. W. Herrlek and Fikentscher, H. to Badische Aniline and Soda Fabrik).
9. Alfrey, T. Jr., Bohrer, J. J. and Mark, H., *Copolymerization* (*High Polymers*, Vol. VIII), Interscience, New York, 1952, p. 5.
10. Finemann, M. and Ross, S. D., *J. Polym. Sci.*, 1950, **5**, 269.

NUCLEAR REDUCTION IN FLAVONOIDS

IN the biogenetic theories to account for the formation and interconversion of flavonoid compounds in nature, nuclear oxidation (introduction of phenolic hydroxyl groups) and nuclear reduction (removal of phenolic hydroxyl groups) have been definitely shown to occupy an important place[1, 2]. Nuclear oxidation of phenolic compounds both with alkaline persulphate and with alkaline hydrogen peroxide (through an *ortho*-hydroxy-aldehyde system) has been taken as a laboratory model for the biogenetic introduction of phenolic hydroxyl group in these compounds and the typical conversion of 3, 7, 3′, 4′-O-tetramethylquercetin into 3, 7, 3′, 4′-O-tetramethylgossypetin[3] has been invoked as a valid proof for the hypothesis that the former is a natural precursor for the latter. However, the reversal of this process (removal of phenolic hydroxyl group), *viz.*, nuclear reduction, has remained for a long time only as a hypothesis without laboratory parallel until a number of such nuclear reductions were accomplished in the laboratory[4–7] and the method involves the preparation of the phenolic tosylates and their subsequent hydrogenolysis using Raney nickel.

In the present communication, we wish to report the successful conversion of a gossypetin derivative into a quercetin derivative by the process of nuclear reduction. This would eventually close the hitherto unfilled gap in the biogenetic hypothesis of the formation of quercetin from gossypetin with an experimental support.

The necessary starting material for this purpose is a suitable gossypetin derivative in which the 8-hydroxyl group is free. Since gossypin (the 8-glucoside of 3, 5, 7, 8, 3′, 4′-hexahydroxyflavone) is known to occur in the flower petals of *Hibiscus vitifolius*[8], it was decided to procure the starting material from this source. However, extraction of fresh flower petals of this plant yielded a mixtue of gossypin[8] and the 8-O-glucuronide of gossypetin[9] which could be separated on a cellulose column. The structure of the two pigments could be confirmed individually by the technique of methylation and hydrolysis whence the former gave 3, 5, 7, 3′, 4′-O-pentamethylgossypetin and glucose while the latter afforded the same flavone derivative and glucuronic acid. Enzymatic hydrolysis, using the enzymes (β-glucosidase as well as β-glucuronidase), and also U.V. spectral data with and without the addition of the various shift reagents confirmed these conclusions. Since in the above two glycosides, only the 8-hydroxyl group is blocked, the mixture of the two compounds could directly be used for our studies without any need to separate the constituents.

A suspension of the dry and finely powdered glycosidic mixture (1 g), isolated from the fresh flower petals of *H. vitifolius*, in dry acetone (200 ml) was methylated with freshly distilled dimethyl sulphate (8 ml) and ignited potassium carbonate (20 g) by refluxing the mixture for 50 h (negative ferric reaction). The methylation product (1 g) could not be satisfactorily crystallized and was directly hydrolyzed by refluxing with aq. H_2SO_4 (7%; 50 ml) for 2 hr. The hydrolyzate, on cooling in ice, slowly deposited 8-hydroxy-3, 5, 7, 3′, 4′-pentamethoxyflavone (700 mg), m.p. 196–198° (lit.[10] m.p. 196–198°); acetate (Ac_2O and pyridine), m.p. 215–216° (lit.[10] m.p. 215–216°).

Tosylation of the above partial methyl ether of gossypetin using the hydroxy compound (500 mg), crystallized toluene *p*-sulphonyl chloride (400 mg) and ignited potassium carbonate (5 g) in dry acetone (150 ml) medium gave 3, 5, 7, 3′ 4′-pentamethoxy-8-tosyloxyflavone as colourless plates (from alcohol) (200 mg), m.p. 144-146° (Found : C, 59·6; H, 4·7; $C_{27}H_{26}O_{10}S$ requires C, 59·8; H, 4·8%).

The above tosyl ester was subjected to nuclear reduction as follows : To a clear solution of the tosyl ester (200 mg) in ethanol (500 ml), Raney nickel catalyst (2 g) was added and purified H_2 gas was bubbled through the suspension of the above, with stirring, for 5 hr. After filtration, the residue was washed repeatedly with ethanol and the combined ethanolic solution evaporated. The residue, obtained after removal of the alcohol, was taken in benzene and the benzene solution was washed with 1% aq. NaOH solution and then with water. The organic layer was dried (anhyd. Na_2SO_4) and evaporated when a residue was obtained which was separated by T.L.C. over silica gel plates using toluene-ethyl acetate-formic acid (5:4:1) as the irrigating solvent. The slow-moving band, on elution with methanol, yielded crystalline pentamethylquercetin[11] (3, 5, 7, 3′, 4′-pentamethoxyflavone) (50 mg), m.p. and mixed m.p. 151-152°. Thus starting from a derivative of gossypetin, a quercetin derivative could be prepared in the laboratory.

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1. Seshadri, T. R. *Experientia* (Supplement II), 1955, p. 258.
2. —, *Tetrahedron*, 1959, 6, 169.
3. Rao, K. V. and Seshadri, T. R., *Proc. Indian Acad. Sci.*, 1947, **25A**, 417.
4. Jain, A. C. and Seshadri, T. R., *J. Sci. Industr. Res., India*, 1953, **12B**, 503.
5. Ramanathan, K. and Venkataraman, K., *Proc. Indian Acad. Sci.*, 1953, **38A**, 40.
6. Jain, A. C. and Seshadri, T. R., *Ibid.*, 1953, **38A**, 294 and 417.
7. Murti, V. V. S., Raman, P. V. and Seshadri, T. R., *Indian J. Chem.*, 1966, **4**, 396.
8. Rao, K. V. and Seshadri, T. R., *Proc. Indian Acad. Sci.*, 1946, **24A**, 352.
9. Nair, A. G. R. and Subramanian, S. S., *Indian J. Chem.*, 1974, 12, 890.
10. Rao, K. V. and Seshadri, T. R., *Proc. Indian Acad. Sci.*, 1946, **24A**, 375.
11. Heilbron I. and Bunbury, H. M., *Dictionary of Organic Compounds*, 4th Edn., Vol. 5, Eyre & Spottiswoode, London, 1965, p, 2832.

DIPOLE MOMENT AND INTRAMOLECULAR HYDROGEN BONDING IN ORTHO-SUBSTITUTED PHENOLS

WE wish to report here certain interesting features of the dipole moments of some ortho-substituted phenols and anisoles and the corresponding para-substituted compounds and also an explanation for them. It may be seen from Table I that the difference between the dipole moments ($\Delta\mu$) of an ortho-substituted anisole and the corresponding ortho-substituted phenol is large compared to a similar difference for the corresponding para derivatives.

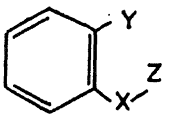
The ortho-halogenophenols and ortho-nitrophenol exist to a larger extent in the *s-cis* configuration (I) due to the stabilization by intramolecular hydrogen bonding; this leads to a lower dipole moment in these compounds. [*cf.* Table I. The calculated dipole moment of a *cis*-form is smaller than the *s*-trans form (II) in these compounds[1]]. On the other hand in the ortho-substituted anisoles the introdction of a substituent Y into the ortho-position of the benzene nucleus leads to considerable steric hindrance for the *s-cis* orientation which becomes energetically less favourable and the *s*-trans orientation predominates in almost all the ortho-substituted anisoles. The large contri-

TABLE I

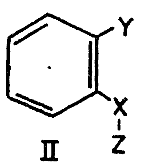
Dipole moments of phenols and anisoles and the extent of s-cis *form in phenol and* s-*trans* *form in anisoles*

Substituent	$\mu_{obs.}$ for phenol (μ_P) (D)	$\mu_{obs.}$ for anisole (μ_A) (D)	($\mu_A - \mu_P$) = $\Delta\mu$ (D)	% of intramolecular H-bonding in phenol (*s-cis* form[a])	% of *s*-trans form in anisole
o-F	1·30[a]	2·31[c]	1·01	85	82[c]
p-F	2·17[a]	2·04	−0·13		
o-Cl	1·31[a]	2·50[c]	1·19	87	90[c]
p-Cl	2·30[a]	2·26	−0·04		
o-Br	1·39	2·47[c]	1·08	84	90[c]
p-Br	2·27	2·26	−0·01		
o-NO_2	3·12[a]	4·83[d]	1·71	100	94[d]
p-NO_2	5·05	4·80	−0·25		
o-CHO	2·88[b]	4·21[b]	1·33	..	..
p-CHO	4·21	3·73	−0·48	..	..
o-$COCH_3$	2·81	4·02	1·21	..	..
p-$COCH_3$	3·95	3·56	−0·39		

a, *b*, *c* and *d* refer to values from references 1, 2, 3 and 4 respectively. Other values are from reference 5.

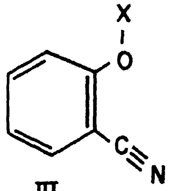


I
s-Cis



II
s-Trans

Y = Substituent
X = O
Z = H or CH_3



X= H or CH_3

III

bution of the *s*-trans configuration leads to a higher dipole moment[2-4] in the ortho-substituted anisole. (The calculated dipole moment of a *s*-trans form is larger than a *cis*-form in these compounds). Because of the larger moment of the ortho-substituted anisole and the lower moment of the corresponding phenol due to intramolecular hydrogen bonding, the difference between the two dipole moments is large. The difference between the dipole moments of a para-substituted anisole and the corresponding phenol is not significant because of the free rotation of the groups.

When the extent of intramolecular hydrogen bonding in an ortho-substituted phenol decreases (due to solvent effect), then the s-trans orientation will contribute more and this will result in a higher moment of the phenol. Hence the difference between the dipole moments of the ortho-substituted anisole and phenol should decrease. The dipole moment[2] of salicylonitrile in carbon tetrachloride, benzene and dioxane is 3·2, 4·38 and 5·03 D respectively. This nitrile exists in the *s-cis* form to the extent of 83% in carbon tetrachloride[2] and 43% in benzene[2] and almost exclusively in the *s*-trans form in dioxane[2]. The observed moment of *o*-methoxybenzonitrile indicates that it exists almost in the s-trans form III ($\mu_{obs.}$ = 4·97 D; $\mu_{calc.}$ = 4·8 D). The difference between the dipole moments of *o*-methoxybenzonitrile and salicylonitrile in carbon tetrachloride, benzene and dioxane are 1·77, 0·59 and 0·00 D respectively and this is the order expected from the extent of intramolecular hydrogen bonding.

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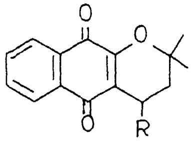
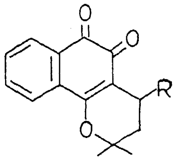
1. Baliah, V. and Uma, M., *Ind. J. Chem.*, 1972, **10**, 395.
2. Curran, B. C. and Chaput, E. P., *J. Am. Chem. Soc.*, 1947, **69**, 1134.
3. Anzilotti, W. F. and Curran, B. C., *ibid.*, 1943, **65**, 607.
4. Dryden, J. S. and Meakins, B. J., *Proc. Phys. Soc.*, 1956, **69B**, 252.
5. McClellan, A. L., *Tables of Experimental Dipole Moments*, W. H. Freeman and Co., San Francisco, 1963.

SYNTHESIS OF 4-HYDROXY-α-LAPACHONE AND ITS β-ISOMER

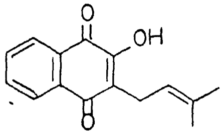
Recently Duarte Weinberg *et al.*[1] reported the isolation of 4-hydroxy-α-lapachone (I) along with other compounds from the trunk wood of *Zeyhera tuberculosa* (Vell) Bur. The assigned structure was based on chemical and spectral studies. We have now carried out the synthesis of 4-hydroxy-α-lapachone (I) and its β-isomer *viz.*, 4′-hydroxy-β-lapachone (II) starting from lapachol (III). The synthesis involves the successful extension of the allylic acetoxylation first carried out by Barton *et al.*[2] during the course of synthesis of β-amyrin using N-bromosuccinimide and lead tetraacetate. Recently Talapatra *et al.*[3] reported the failure of this reaction when carried out with dihydroxanthyletin.



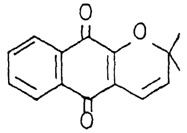
I R=OH
IV R=H
VI R=OAc



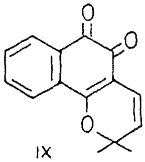
II R=OH
V R=H
VIII R=OAc



III



VII



IX

Lapachol (III) was cyclised with HCl in glacial acetic acid and conc. H_2SO_4[5] separately to give α-lapachone (IV) and β-lapachone (V) respectively IV when refluxed with N-bromosuccinimide and lead tetraacetate in dry benzene furnished 4-acetoxy-α-lapachone (VI) in good yields. VI was then hydrolysed with dil. HCl to give 4-hydroxy-α-lapachone (I). The structure of the compound I was confirmed by converting it into dehydro-α-lapachone (VII) with *p*-toluene sulphonic acid and dry benzene. For comparison the authentic sample of the compound VII was prepared by the dehydrogenation of α-lapachone with DDQ[6]. Further the compound VII was also obtained by dehydrogenation of I with conc. HCl. I on acetylation gave compound VI. The compound I and its acetate (VI) agreed with the natural sample of I and its acetate (VI) respectively on TLC. The IR, UV and NMR spectra of I and VI agreed with the reported values[1] of the natural sample of I and its acetate (VI).

β-lapachone (V) was similarly refluxed with N-bromosuccinimide and lead tetraacetate in dry benzene to give 4′-acetoxy-β-lapachone (VIII) as major product. VIII was hydrolysed with dil. HCl to give II. The structures of II and VIII were assigned on the basis of spectral and chemical studies.

II when dehydrated with *p*-toluenesulphonic acid in dry benzene gave VII, instead of expected dehydro-β-lapachone (IX). The latter is very prone to rearrange to the α-isomer (VII), especially if a trace of acid is present[6].

Details of this work will be published elsewhere.

The authors wish to express their grateful thanks to Professor G. G. de Oliveira for the supply of natural sample of 4-hydroxy-α-lapachone and its acetate. One of the authors (R. B. G.) is grateful to the Centre of Advanced Studies in Chemistry, Department of Chemistry, University of Delhi, for the award of a fellowship.

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1. Duarte Weinberg, M. D. L., Gottlieb, O. R. and Oliveira, G. G., *Phytochemistry*, 1976, **15** (4), 570.
2. Barton, D. H. R., Lier, E. F. and McGhie, J. F., *J. Chem. Soc., C*, 1968, p. 1031.
3. Talapatra, B., Mukhopadhyay, S. K., Chaudhuri, M. K. and Talapatra, S. K., *Indian J. Chem.*, 1976, **14** (**B**), 129.
4. Gupta, S. R., Malik, K. K. and Seshadri, T. R., *Ibid.*, 1969, **7**, 457.
5. Hooker, S. C., *J. Chem. Soc.* (*Trans.*), 1892, **61**, 611.
6. Burnette, A. R. and Thomson, R. H., *J. Chem. Soc., C*, 1967, p. 1261.

PHOTOOXIDATION OF PTEROSTILBENE FROM *PTEROCARPUS MARSUPIUM* ROXB.

Pterocarpus marsupium Fam. Leguminosae is a commercial timber available in the Telangana region of Andhra Pradesh (India), known to be resistant to termites and used in the treatment of diabetes[1]. We now report the isolation of pterostilbene from *Pterocarpus marsupium* and the photooxidation of its derivatives.

The heartwood of this plant on extraction with petroleum ether (60–80°) gave 0·04% of a colourless compound (I) with m.p. 87°. Oxidation of its O-acetyl derivative (II) (m.p. 135°)[2] with potassium permanganate in acetone gave *p*-acetoxy benzoic acid (m.p. 185°)[2] and 3, 5-dimethoxy benzoic acid (m.p. 184°)[2]. Its m.p., its superimposable IR spectrum and its undepressed m.p. with an authentic sample of pterostilbene confirmed it to be pterostilbene (I).

The I.R. spectrum of pterostilbene (I) and its O-acetate (II) in $CHCl_3$ showed an absorption around 965 cm^{-1} attributable to trans $-CH=CH-$[3]. The U.V. spectrum of (II) in CH_3OH gave λ max (log. ϵ, 226 nm (3·06); 242 nm (3·63) and 308 nm (3·98) similar to that of trans stilbene[4]. Thus (I) appears to be a trans stilbene.

Photolysis of O-methyl pterostilbene (*III*): O-Methyl pterostilbene (III) (m.p. 57°)[2] was dissolved in purified cyclohexane ($3 \cdot 7 \times 10^{-3}$ M) containing 5 mole-% of resublimed iodine and irradiated using medium pressure mercury lamp for 36 hr. The use of iodine in the photooxidation of synthetic stilbenes is well known.[5] At the end of the reactiion period the solvent was removed under reduced pressure. The residue, on column chromatography using silica gel as adsorbant and benzene-petroleum ether (60–80°) (9 : 1) as eluent gave two products. One of the two products was considered to be a polymerised material which was not further investigated. The other product obtained in 20% yield with m.p. 103° was chracterised to be 2, 4, 6-trimethoxy phenanthrene (IV) on the basis of lhe following evidence.

The mass spectrum of (IV) shows M^{+} at *m/e* 268 as a base peak and together with elemental analysis (C, 76·11%; H, 5·96%) the moecular formula was found to be $C_{17}H_{16}O_3$.

The I.R. ($CHCl_3$) of (IV), ν_{max} 1620, 1601, 1570 (aromatic), and 840 cm^{-1}, U.V. (CA_3OH) λ_{mxz} (log ϵ) 240 nm (3·14), 263 nm (3·84), 293 nm (3·01) and 326 nm (2·70) are characteristic of phenanthrene[6], NMR (60 MHz) $CDCl_3$, TMS, δ 3·80, 3·86 and 3.98 (s, 3H each, three-OCH_3), δ 6·60 (*d*, $J = 3$Hz, 1H, C_3–H), δ 6·73 (*d*, $J = 3$Hz, 1H, C_1–H), δ 8·92 (*d*), $J = 3$Hz, 1H, C_5–H), and δ 7·10–7·70 (*m*, 4H, aromatic). It is reported[7] that C_4–H and C_5–H in phenanthrene appear downfield. Hence the proton δ 8·92 is assigned to C_5–H of the photo-product (IV).

Photooxidation of O-methyl pterostilbene (III) consists of first isomerisation[5] to dihydrophenanthrene (V) which then undergoes oxidation in the presence of iodine to 2, 4, 6-trimethoxy phenanthrene (IV).

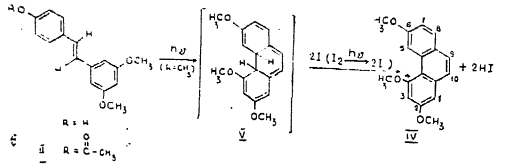

CHART-I

The mass spectral fragmentation pattern also confirms the phenanthrene structure for (IV). The loss of $-CH_3$ and $-CO$ units appears to be characteristic of methoxy substituted phenanthrenes. m/e 268 (100%) $\xrightarrow{-CH_3}$ 253 (10%) $\xrightarrow{-CO}$ 225 (23%) $\xrightarrow{-CH_3}$ 210 (10%) $\xrightarrow{-CH_3}$ 195 (10%) $\xrightarrow{-CO}$ 167 (10%) $\xrightarrow{-CO}$ 139 (20%). The prominent ion m/e 149 (47%) arising from the parent ion by the symmetrical cleavage by losing 119 units, may be represented by the structure (VI) (Chart II).

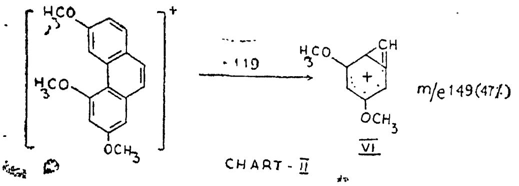

CHART - II

Photolysis of pterostilbene (I) under similar conditions described for (III) gave a mixture of four compounds as shown by tlc using benzene-petroleum ether (60–80°) (9 : 1) as solvent system, and the characterisation of these compounds is under investigation. O-Acetylpterostilbene (II) was not photooxidised on irradiating it for longer duration (72 hr) and also passing oxygen. The failure of synthetic stilbenes carrying electron withdrawing groups like $-NO_2$ and $-OCOCH_3$ to undergo photooxidation was earlier noticed and this failure is attributed to inter-system crossing.[5]

The authors wish to thank Prof. T. Navaneeth Rao, Head of the Department of Chemistry for providing facilities. Our thanks are also due to Prof. F. E. King, University of Nottingham for authentic sample of pterostilbene and Prof. M. R. Suxena, Emeritus Professor, Department of Botany, Osmania University, for providing the plant material.

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1. Seshadri, T. R., "Polyphenols of pterocarpus and Dalbergia Wood (Review article)," *Phytochemistry*, 1972, **11**, 881.
2. King, F. E., Cortell, C. B., Godson, D. H., Jurd, L. and King T. J., *J. Chem. Soc.*, 1953, p. 3693.
3. Kariyone, T., *et al.*, *Pharmaceutical Soc., Japan*, 1959, **79**, 219. Yamaguchi, K., *Spectral Data of Natural Products*, Elsevier Publ. Co., 1970, **1**, 61.
4. Phillips, J. P., *Spectra-structure Correlation.* Pub. Academic Press (N.Y.), 1964, p. 63.
5. Mellory, F. B., Wood, C. S., Gordon, J. T., Lindquist, L. C. and Savitz, M. L., *J. Am. Chem. Soc.*, 1962, **84**, 4361.
6. Newman, M. S. and Hussey, A. S., *Ibid.*, 1947, **69**, 3025.
7. Jonathan, N., Gordon, S. and Dalley, B. P., *J. Chem. Phy.*, 1962, **36**, 2443.

CONTRACTION OF AMPHIBIAN SKELETAL MUSCLE BY COMPETITIVE NEUROMUSCULAR BLOCKING AGENTS

[illegible]

[illegible]

[illegible]

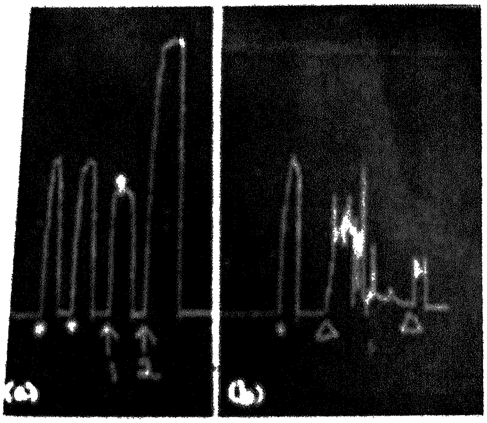

FIG. 1. Showing the action of neuromuscular blocking agents in high concentrations. [illegible] white dot [illegible] of ACh, at 1 and 2, 10 and 20 [illegible] of curare respectively. [illegible] 10 and 20 [illegible] of gallamine respectively.

ACh. At the height of contraction ACh was devoid of any effect, whereas, at the height of a submaximal contraction by ACh, curare produced a further contraction of less amplitude. This effect of curare was not blocked by equal concentrations of atropine and pentolinium. Gallamine, produced fibrillatory twitches [illegible], at 0.1, 1, 10 and 20 mg concentration. Occasionally it also produced a smooth contraction reaching its peak in 7–10 minutes. A tissue once exposed to gallamine failed to respond to next higher concentration with a dose related response. Atropine, pentolinium and curare blocked the action of gallamine.

Curare induces contraction of the chronically denervated mammalian skin by an action outside the end plate zone. In the present study, curare induced contraction of skin and gallamine induced fibrillatory twitches, inspite of their own neuromuscular blocking action, indicating, that in amphibian skin also the effect is produced by an action outside the end plate zone. But the effect of gallamine appears to be on the muscle fibre, as it has induced fibrillatory twitches showing the incoordinated contraction of muscle fibres. "Strychnine like reactions" in animals, dying of arrow poison and with crude preparations of curare, has been reported[6]. The present findings raise the possibility of convulsions occurring owing to an action of curare outside the neuromuscular junction.

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1. Acharya, S. R. K. and Govinda Rao, A. R., *Arogya J. Health Sci.*, 1975, 1 (1), 69.
2. Burn, J. H., *Practical Pharmacology*, Blackwell, Oxford, Scientific Publications, 1952, p. 2.
3. Deshpande, V. R., Kharkar, V. T., Deshpande, A. M. and Paranjape, B. D., *Ind. J. Pharmac.*, 1976, **8** (1), 39.
4. Diplama, J. R. Drills, *Pharmacology in Medicine*, McGraw Hill Book Company, New York, 1958, p. 551.
5. Goodman, L. S. and Gilman, A., *The Pharmacological Basis of Therapeutics*, Collier MacMillan Limited, London, 1970, p. 606.
6. Feldman, S. A., *Muscle Relaxants*, W. B. Saunders Company Ltd., London, 1973, p. 3.
7. Sollman, T. A., *Manual of Pharmacology*, W. B. Saunders Company Ltd., London, 1957, p. 447.

POLAROGRAPHIC INVESTIGATION OF CADMIUM(II)-MIXED LIGAND SYSTEM

In continuation of our earlier studies[1-4], polarographic investigation of Cd(II)-malic acid-thiocyanate system is described in the present paper.

Experimental

The chemicals used in the present investigation were of Analar quality. Potassium malate and potassium thiocyanate used as complexing agents were in neutral forms. Appropriate quantities of potassium nitrate were added to the solution which were polarographed to maintain constant ionic strength at 2·5 M and in the pH range of 6·6 to 6·8. Purified nitrogen was passed to remove the air.

Half-wave potentials ($-E_{\frac{1}{2}}$) and diffusion currents (μ_A) were recorded on a Cambridge Pen Writing Polarograph. The mercury electrode had the following characteristics:

$t = 3 \cdot 33$ sec. in 0·5 M KCl (open circuit)
$m = 1 \cdot 75$ mg/sec, $m^{2/3} \times t^{1/6} = 2 \cdot 15 \text{ mg}^{2/3} \text{ sec}^{-1/2}$

and these were maintained throughout the investigation. The measurements were carried out at 25 ± 0·5° C. The potential measurements were made with respect to saturated calomel electrode (S.C.E.) having a negligible cell resistance, with the mercury head kept at a height of 60 cm. The system was standardized against Cd(II)-malate and Cd(II)-thiocyanate. The experimental conditions were controlled by running parallel experiments in Cd(II)-thiocyanate and Cd(II)-malate systems. The experimental stability constants of these systems agreed reasonably well to those reported earlier[5-8].

In the mixed ligand systems, the concentration of thiocyanate ion (SCN) was held constant at 0·16 and 0·40 M because at these concentrations 1:1 and 1:2 Cd(II)-thiocyanate complex species were found to be in predominant quantities.

In the first set, polarograms were recorded of all the solutions, containing 1 mM Cd(II), after adding the requisite amount of potassium nitrate to adjust ionic strength at 2·5 M, and keeping 0·16 M potassium thiocyanate constant and varying concentrations of malate ions. The same procedure was adopted in the second set in which thiocyanate ion concentration was kept at 0·40 M.

Results and Discussion

In all the systems, a well defined, diffusion controlled, single wave was observed. The linear relationship between $E_{d.e.}$ *vs* log *i*/*id*−*i* with slope values in the range of 30–32 mV indicated a reversible reduction involving two elections.

The shift in half-wave potential of Cd(II) ion in the presence of the mixture of thiocyanate and malate ions is greater than that with the malate ion alone. This suggests the formation of Cd-MAL-SCN mixed complexes (Fig. 1).

The method of Schaap and McMasters[9] was applied to evaluate the constnts A, B, C and D in both sets. The values of these constants are;

At 0·16 M [SCN]:

log A = 1·00, log B = 2·53 ± 0·03,
log C = 2·78 ± 0·04 and log D = 3·68 ± 0·03.

At 0·40 M [SCN]:

log A = 1·56, log B = 2·58 ± 0·01,
log C = 3·00 ± 0·02 and log D = 3·93 ± 0·03.

The determination of composition and the evaluation of stability constants of the mixed ligand complexes show that there is a positive replacement of thiocyanate ions by malate ions in the Cd(II)-malate-thiocyanate system. Again the fact, that the mean value of coefficient D coinciding with stability constant β_{30} for $[Cd(MAL)_3]^{4-}$ complex in the Cd-MAL-SCN system points to the same conclusion. Here log $\beta_{30} = 3 \cdot 54 \pm 0 \cdot 03$ whereas mean value of log D is 3·80 ± 0·03.

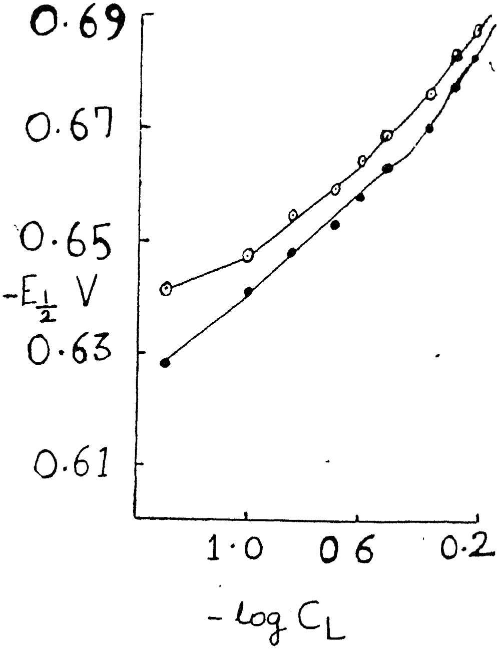

FIG. 1. Plots of $-E_{\frac{1}{2}}$ 'V' against − log [MAL]. for cadmium(II)-malate-thiocyanate system.
1. ● at 0·16 M [SCN] and
2. ⊙ at 0 40 M [SCN].

The stability constants of the mixed ligand species were calculated from the constants A, B, C and D and were log $\beta_{21} = 3 \cdot 14$ and log $\beta_{11} = 2 \cdot 98$.

Following the procedure of Watters *et al.*[10], the predicted values for the complexation constant for $[Cd(MAL)_2 (SCN)]^{3-}$ was $3 \times \beta_{20}^{2/3} \times \beta_{03}^{1/3} = 10^{2.54}$, while the observed value was $10^{3.14}$. From this enhancement value is obtained as $10^{0.60}$. The complexaation constant for mixed complex species $[Cd(MAL)(SCN)]^{-}$ is $2 \times \beta_{20}^{1/2} \times \beta_{02}^{1 \cdot 12} = 10^{2.47}$ as compared to those observed $10^{2.98}$, with the enhancement of $10^{0.51}$.

It is observed that the enhancement is more for $[Cd(MAL)_2 (SCN)]^{3-}$ than for $[Cd(MAL)(SCN)]^{-}$. This enhancement may be due to the cooperative effects of electrostatic forces between different kinds of ligands and steric effects. This also supports our data that the enhancement values are greater in the case of Cd(II)-malate as compared to Cd(II)-malonate thiocyanate and Cd(II)-succinate-thiocyanate mixed systems. This clearly indicates that the stability constants of mixed ligand complexes decrease as the size of the organic ligands increases. This gradual decrease in the stability constants may be due to the steric hindrance.

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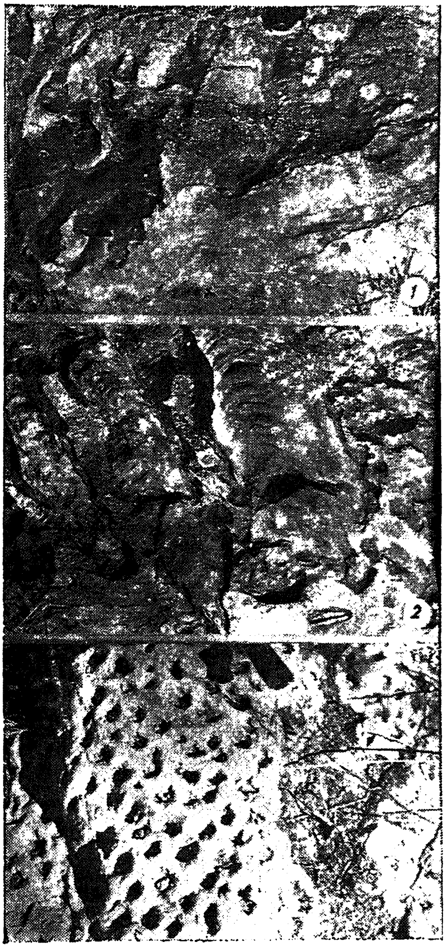
1. Bidkar, R. G., Dhuley, D. G. and Bhobe, R. A., *Curr. Sci.*, 1976, **45**, 168.
2. Jadhav, P. D., Bidkar, R. G., Dhuley, D. G. and Bhobe, R. A., *J. Ind. Chem. Soc.*, 1976, **53**, 451.
3. — and Bhobe, R. A., *J. Inorg. Nucl. Chem.* (in press).
4. Bidkar, R. G., Dhuley, D. G. and Bhobe, R. A., *Indian J. Chem.* (in press).
5. Topuzovski, B., *God. Zb. Prir. Mat. Fak. Univ. Skopje. Mat. Fiz. Hem.*, 1972, **22**, 237.
6. Khurana, S. C. and Gupta, C. M., *Talanta*, 1973, **20** (8), 789.
7. Dhoot, L. N. and Karmalkar, P. K., *Vikram Univ. J.*, 1960, **4**, 81.
8. Humffray, A. A., Bond, A. M. and Forrest, J. S., *J. Electroanalyt. Chem.*, 1967, **15**, 67.
9. Schaap, W. B. and McMasters, D. L., *J. Am. Chem. Soc.*, 1961, **83**, 4699.
10. Watters, J. I. and DeWitt, R., *Ibid.*, 1960, **82**, 133

STROMATOLITES AND PHOSPHORITE IN THE TIROHAN LIMESTONE OF CHITRAKUT AREA, DISTRICT SATNA, M.P.

DURING a recent visit to Chitrakut area the association of stromatolites and phosphorite was noticed for the first time from the Tirohan limestone in the river bed near Sitakund about 2 km from Chitrakut on the Chitrakut-Satna road. The Tirohan Limestone is considered as the youngest formation of the Semri Group (Semri Series of Auden, 1933[1]) and has been correlated with the Rohtas limestone of Son Valley area. The stratigraphic succession is given in Table I.

TABLE I

Upper Vindhyan	Kaimur Sandstone
	.... Unconformity ....
Semri Group	Tirohan Limestone
	Upper Glauconitic Sandstone
	Pellet Limestone
(Lower Vindhyan)	Lower Glauconitic Sandstone
	Basal Conglomerate
	.... Unconformity ....
	Bundelkhand Granite



FIGS. 1–3. Fig. 1. *Baicalia baicalica*. Tirohan Limestone,. Loc. near Sitakund, Chitrakut area, M.P. Fig. 2. *Colonella columnaris* with black encrustation of phosphorite, Loc. near Sitakund, Chitrakut area, M.P. Fig. 3. Transverse section of *Colonella columnaris* giving mesh-like appearance. Same locality as above.

Kumar (1976[3]) has recorded algal mats and faintly preserved stromatolites from the Tirohan limestone of Chitrakut area. This note records for the first time well-developed columnar stromatolites *Baicalia baicalica* (Fig. 1) and *Colonella columanaris* (Figs. 2, 3) from the Tirohan limestone in which the former dominates over the latter. Both isolated and crowded colonies are recorded. Due to over-crowding of the colonies of *Colonella columnaris*, on weathering, the rock attains a mesh-like appearance in transverse section. Both these forms resemble those described from the Gangolihat Dolomite from Chandak area, Pithoragarh District, U.P., by Valdiya (1969)[4]. However, the size of the colonies in the present area is comparatively small.

Around some of the stromatolite columns are thin black to dark greyish black phosphorite encrustations (Fig. 2) which are made up of cellophane. Besides these encrustations, phosphorite is also present as small lenticles and stringers. In intraformational conglomerate band, the phosphorite is seen as pellets in carbonate matrix. Some of the pellets show internal structure resembling intestinal markings. The phosphorite encrustation gives 52·8% P_2O_5 content.

The sedimentary structures recorded from the Tirohan limestone are parallel lamination with low angle discordances, small scale ripple lamination, and intraformational conglomerate. Associated with these are oncolites. All these suggest intertidal to supratidal environment of deposition.

The association of phosphorite and stromatolite is rare but quite interesting. Similar association has also been reported from the Gangolihat Dolomites of Pithoragarh, U.P. (Valdiya 1969[5]) and from Aravalli Formation of Udaipur, Rajasthan (Banerjee 1971[2]). It may be pointed out that in all these associations of phosphorite and stromatolites, there is one thing common, *i.e.*, the assemblage of *Baicalia* and *Colonella*. The age of Gangolihat Dolomites, Aravalli Formation and Tirohan limestone (equivalent of Rohtas limestone) has been taken as Middle Riphean (Valdiya 1969[4], Banerjee 1971[2] and Kumar 1976[3]). Almost the same age and the same assemblage in such widely separate regions cannot be taken as a mere coincidence but may indicate some relationship between the stromatolite assemblage and occurrence of phosphorite.

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1. Auden, J. B., *Mem. Geol. Surv. India*, 1933, p. 57.
2. Banerjee, D. M., *Bull. Geol. Soc. Am.*, 1971, 82, 2319.
3. Kumar, S., *Jour. Palaeo. Soc. India*, 1976, **19**, 24.
4. Validiya, K. S., *Jour. Geol. Soc. India*, 1969, **10**, 1.
5. —, *Curr. Sci.*, 1969, **28** (17), 415.

A NOTE ON THE "INTRAFORMATIONAL RECUMBENT FOLD" AN UNUSUAL BEDDING TYPE IN THE JABALPUR SANDSTONE, JABALPUR AREA, M.P.

JABALPUR beds are of Jurassic age and are well known for their clay deposits. They underlie the marine Lameta Beds. The total thickness of Jabalpur clays and sandstone is about 10 m and the lithology varies from coarse grained sandstones t clays. The stratigraphic succession of the sedimentar sequence is given in Table I (Matley 1921[1]).

TABLE I

Deccan Traps	Upper Sands
	Upper limestone
Lameta Beds	Mottled Nodular beds
	Lower Limestone
	Green Sands
Jabalpur Group	Sandstones and Shales

A rare but quite characteristic sedimentary structure referred to as intraformational recumbent folds, is recorded for the first time from the Jabalpur Sandstones in the Railway cutting about ½ km from the Railway Station towards Bara Simla.

The intraformational recumbent folds are a form of large scale cross bedding, in which the foreset laminae are overturned in their upper parts (Fig. 1). The strong sediment laden currents, when they flow across a top of the sediment surface exert a strong drag effec Due to this, the underlying set of foreset laminae i deformed and dragged to produce folds which are overturned and termed as intraformational recumbent fold by McKee *et al.* (1962)[2].

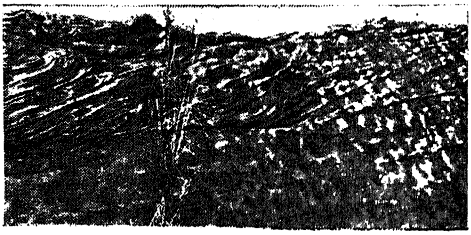

FIG. 1. Intraformational recumbent folds in th Jabalpur Sandstone.

This sedimentary structure has been commonl observed in fluviatile deposits (Reineck and Singh 1973)[3] and has been reported from point bars and delt front environment of Mississippi River, flood plai deposits of Colorado River and Channel bar deposit of Brahmaputra River.

The arkosic sandstones and clays of the Jabalpu Beds are considered as of freshwater origin. Apa from intraformational recumbent folds, the oth sedimentary structures are bar cross bedding, larg scale cross bedding, parallel lamination and mud gal All these sedimentary features confirm that these san stones are of fluvial origin, possibly a point bar deposit.

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---

1. Matley, C. A., *Rec. Geol. Surv. India*, 1921, **53**, 142.
2. McKee, E. D., Reynolds, M. A. and Baker, C. H., *U.S. Geol. Surv. Profess. Papers*, 1962, 450-D.D. 151.
3. Reineck, H. E. and Singh, I. B., *Depositional Sedimentary Environments*, Springer-Verlag, 1973.

DIEL VARIATIONS IN THE ACTIVITY OF ALKALINE AND ACID PHOSPHATASES IN THE SLUG, *LAEVICAULIS ALTE* (FERUSSAC, 1821)

THERE is considerable amount of literature concerning the different types of rhythms in molluscs[1–4]. But very little is known about the diel variations of enzyme activities in these animals. Rhythmic variations in enzymatic activities have been reported in scorpion[5,6], cockroach[7] and snail[8]. The slugs are reported to be more active during night than during the day-time[9] and hence there could be corresponding variations in the various physiological processes of these organisms. Since not much is known about the rhythmic variations in the enzymatic activities of the slug, it would be of interest to study the activity of alkaline and acid phosphatases which are important in transphosphorylation, metabolism of carbohydrates, phospholipids and phosphoproteins[10]. The pattern of activity of these enzymes may reflect the pattern of utilization of inorganic phosphorus in the various metabolic pathways during the course of 24 hr period.

Slugs, collected locally, were maintained in the laboratory in large wooden boxes filled with mud. It is wetted with water once in two days and the animals were fed *ad libitum* with croton leaves. The laboratory provided a cycle of 12 hr cool, (23° C) dark and 12 hr warm (28-29° C) light cycle for the animals during experimentation. The hepatopancreas, a major metabolic mill, and the foot muscle, an organ actively involved in locomotion, were isolated in cold (5° C) from live slugs of similar size at regular interval of 4 hr (two samples at each time). The tissues were weighed and 1% (W/V) homogenates were prepared in 0·25 M ice-cold sucrose solution in Potter-Elvehjem glass homogenizer and centrifgued at 2500 rpm for 10 min. The supernatant was used for the enzyme assay by the method of Fiske and Subba Row as given in Oser[11]. The experiment was repeated for 3 consecutive days to see whether the pattern of activity remained the same. Protein content was determined by the method of Lowry *et al.*[12], and the enzyme activity was expressed as μ moles of inorganic phosphorus (Pi) per mg protein per hour.

Results presented in Fig. 1 indicate that the activity of phosphatases was greater in hepatopancreas than in foot muscle. The higher enzymic activity in hepatopancreas probably reflects its higher metabolic rate over foot muscle. A similar trend was observed for alkaline phosphatase activity in the scorpion[6].

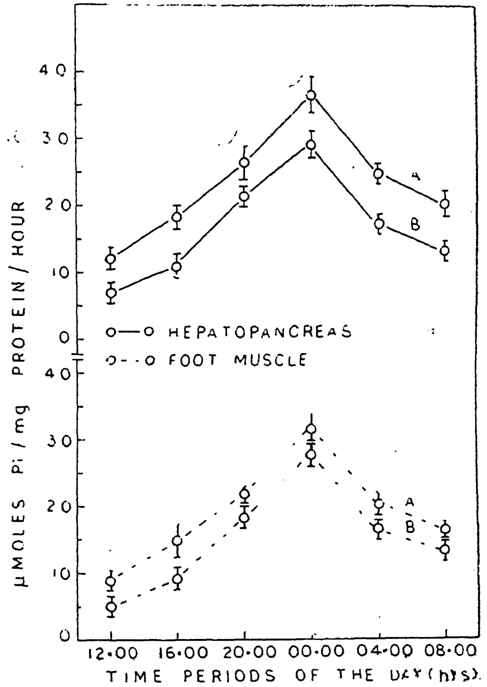

FIG. 1. Rhythmic variations in the activity of alkaline (A) and acid (B) phosphatases in hepatopancreas (o———o) and foot muscle (o——o) of the slug, *Laevicaulis alte*. Values expressed at each time interval are mean ± S.D. of six observations.

It can be seen from Fig. 1 that the alkalne and acid phosphatases exhibited rhythmic variations with maximal activity at 00·00 hr and minimal activity at 12·00 hr of the day. Similar but slightly different pattern of rhythmicity has been reported in the locomotor activity[13], rate of heart beat[5] and electrical activity of the ventral nerve cord[14] of the scorpion. Similar rhythmic variations have also been observed in the acetylcholinesterase (AChE) activity of cockroach[7] and snail[8]. Phosphatases are known to release free inorganic phosphorus from phosphoric acid esters[15] which will be utilized in the formation of high energy phosphate compounds. The elevated activity of phosphatases around 00·00 hr of the day, probably provides the necessary energy for various enhanced physiological processes of the animal at that period. Based on these observations it may, therefore, be suggested that the diel rhythmicity observed in the phosphatases activity might be due to the corresponding variations observed in the physiology of the slug during different periods of the day[9]. The rise and fall observed in the activity of phosphatases between any two consecutive periods of the day are statistically significant.

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1. Dainton, B. H., *J. Exp. Biol.*, 1954, **31**, 165.
2. Brain, M, *Forma Functia*, 1975, **8**, 405.
3. Strumwasser, F. and Wilson, D. L., *J. Gen. Physiol.*, 1976, 67, 691.
4. Pavan Kumar, T. and Sasira Babu K. *Ind. J. Exp Biol* 1976, **14**, 364.
5. Devarajulu Naidu, V. and Padmanabha Naidu, B *Ibid.*, 1976, **14**, 1.
6. Chandra Sekara Reddy, D. and Padmanabha Naidu, B., *Curr. Sci.* 1977, **46**, 147.
7. Vijayalakshmi, S., Murali Mohan, P. and Sasira Babu, K., *J. Insect Physiol.*, 1977, **23**, 195.
8. Murali Mohan, P., Govardhan Reddy, A. V. and Sasira Babu, K., *Curr. Sci.*, 1975, **44**, 347.
9. Kulkarni, A. S. and Nagabhushanam, R., *J. Zool. Soc. Ind.*, 1973, **25**, 71.
10. Chen, P. S., *Adv. Insect Physiol.*, 1966, **3**, 53.
11. Oser, B. L., *Hawk's Physiological Chemistry*, 14th ed., McGraw-Hill Book Company, New York, (1) **1**, 1950.
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
13. Gopalakrishna Reddy, T., Doctoral Dissertation submitted to Sri Venkateswara University, Tirupati, 1966.
14. Venkatachari, S. A. T., *Ind. J. Exp. Biol.*, 1971, **9**, 338.
15. Linhardt, K. and Walter, K., In *Methods of Enzymatic Analysis*, ed. Hans-Ulrich Bergmeyer, Academic Press, New York and London, 1965.

ON THE TRENDS OF INFESTATION OF TWO SPECIES OF *BALIOTHRIPS* UZEL ON PADDY, MAIZE AND THEIR WEED HOSTS

THE role of weeds in relation to the intensity of infestation in crop plants is well known and the observations presented herein relate to *Baliothrips biformis* (Bagnall), a serious pest of rice seedlings in all rice growing areas of the world, particularly in the nursery stage upto 2 to 3 weeks after transplantation, as well as *Baliothrips holorphnus* (Karny)* on young growing maize. In view of the high rate of multiplication, and comparatively short duration of life cycle of *Baliothrips biformis*, with an average of two weeks, severe infestation causes serious damage resulting in the longitudinal curling of leaves which subsequently dry up (Ananthakrishnan, 1971[1], 1973[2]; Grist and Lever, 1969[3]). Information on the population trends of this species prior to transplantation of rice seedlings, as well as that in the weed host of the species, appear to be important criteria in assessing the role of weeds in *Baliothrips biformis* infestations and consequent deweeding to prevent heavy build up of thrips population in the nursery stage. Regular examination of IR 20 seedlings in the nursery stage upto the transplantation period, indicated the beginning of infestation from eighth to tenth day after germination, followed by the build up of populations and then a gradual decline till the twenty-fifth to the twenty-eighth day, when transplantation commenced in the fields under investigation. Two weed species—*Echinochloa colona* and *Cyperus iria*—were abundant, with an estimated density of 221·4/sq. m. and 133·9/sq. m. respectively, along with a sparse distribution of *Cyperus rotundo* all along the bunds, and, the density of paddy seedlings being of the order of 671·8/sq. m.

Analysing the trends of infestation of seedlings, a gradual build up of population was evident, the total population inclusive of adult males, females and immature stages showing the maximum peak during eighteenth to twentieth day and gradually declining thereafter till the time of transplantation. Figure 1 represents the comparative trends of infestation of the adult males, females and immature stages with the peak of immature and adult infestation on the sixteenth and seventeenth days and nineteenth and twentieth days respectively after germination, as was observed in the fields under observation. The adults, more particularly the males, were more abundant than the females with the sex ratio of 3 : 2. The picture of *Baliothrips biformis* in the weeds (while the build up is heavy in the paddy plant) is comparatively low, nevertheless

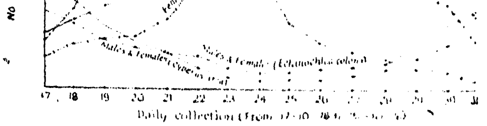

FIG. 1. Trends of infestation of *Baliothrips biformis* on the paddy plant *Oryza sativa* and the weeds *Echinochloa colona* and *Cyperus iria*.

indicating a gradual build up during the period of their decline in the rice seedlings.

Baliothrips holorphnus (Karny) very closely allied to *Baliothrips biformis*, is another potential pest species, (sporadically noted in paddy, but more abundant in the growing seedlings of maize). Evidence of a distinct correlation between the degree of infestation of this species in young growing maize as well as in the abundant weeds, *Borreria hispida* and *Echinochloa colona*, is indicated in Fig. 2.

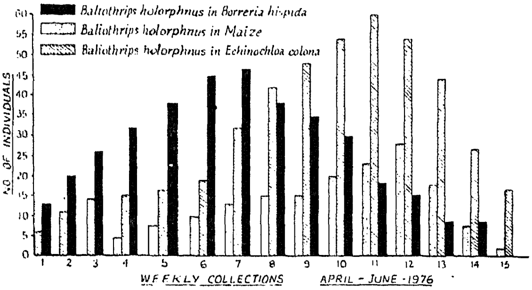

FIG. 2. Trends of infestation of *Baliothrips holorphnus* in maize, *Borreria hispida* and *Echinochloa colona*.

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* Zur Strassen describes this as a new species *Chloethrips blandus* retaining the generic name *Chloethrips* Priesner.

1. Ananthakrishnan, T. N., *J. Scient. and Ind. Res.* 1971, **30** (3), 117.
2. —, *Thrips: Biology and Control*, Macmillan, India, 1973, p. 54.
3. Grist, H. D. and Lever, R. J. A. W., *Pests of Rice: Tropical Science Series*, Longmans Green Co. Ltd., London, 1969, p. 520.

EFFECT OF pH ON THE CELLULAR LOCALIZATION OF Δ^5 3β-HYDROXYSTEROID DEHYDROGENASE IN THE TESTIS OF THE SKINK, *MABUYA CARINATA* (SCHN.)

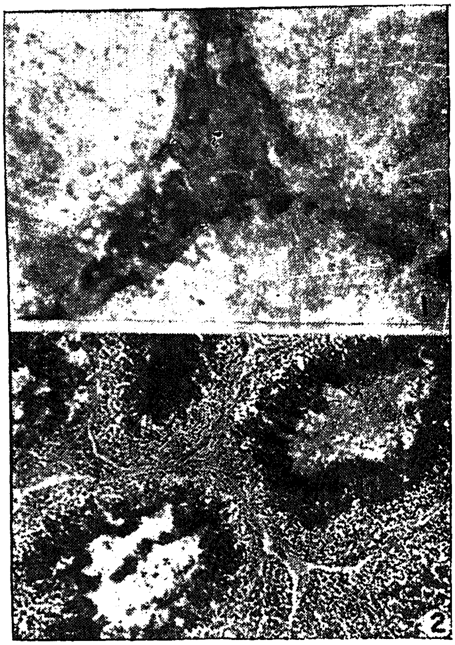
WATTENBERG (1958)[1] was the first to demonstrate the enzyme Δ^5 3β-Hydroxysteroid dehydrogenase (Δ^5 3β-HSDH) histochemically in steroidogenic tissues. Since then, this technique has been widely used as an important tool in the detection of cellular sites of steroidogenesis. The technique involves incubating frozen tissue sections in a buffered medium containing suitable hydroxysteroid substrate, appropriate co-factor and a tetrazolium salt as the final hydrogen acceptor. In the course of our investigations, on the testis of the skink, *Mabuya carinata* (Schn.), we found that the enzyme could be localized in the seminiferous tubules but not in the interstitial cells[2] at pH 7·5 according to the procedure of Baillie *et al.*[3]. But the Leydig cells in the testis of the garden lizard, *Calotes versicolor*[4] and the monitor, *Varanus monitor*[5] gave satisfactory results with the same procedure. This prompted us to undertake a detailed investigation into the kinetics of the localization of Δ^5 3β-HSDH selectively in the interstitial cells of Leydig taking ino account (i) the pH of the buffer, (ii) the steroid substrate concentration, (iii) the co-factor concentration, and (iv) the concentration of the tetrazolium salt.

Testes from the sexually mature skinks during the breeding season were frozen and sections were cut at 16 μ in a cryostat maintained at – 20° C. The sections were thawed for a moment and incubated (with or without prior acetone treatment to fix the enzyme and to remove free lipids) for 1 hr at 37° C. The incubating media consisted of the steroid substrate, Dehydroepiandrosterone or Pregnenolone (0·25 mg to 2·5 mg/ml), β-NAD (0·5 mg to 3 mg/ml) and nitroblue tetrazolium (0·25 mg to 1·5 mg/ml) in 0·1 M Tris buffer at various pHs from 6·5 to 8·5. Controls were incubated omitting the substrate or treating the section in boiling water. After incubation, the sections were fixed in 10% neutral formalin and mounted in glycerine jelly or PVP medium. The enzyme activity was visually quantitated based on the amount of formazan deposition.

The concentration of the co-factor and of the tratrazolium salt were not so critical within the ranges we have used so far as the site of enzyme localisation is concerned. But pH had a profound influence on the activity of the enzyme and on the cellular site of its localization. At precisely pH 8·0, the enzyme showed highest activity and could be selectively localized in the Leydig cells (Fig. 1), whereas at pH 7·5 formazan deposition could be clearly seen mainly in the seminiferous tubules (Fig. 2), and no formazan deposition could be observed at other pH values used. The following alteration of Baillie *et al.*'s incubating medium give best results for this tissue; the steroid substrate (0·4 mg/ml), β-NAD (1·5 mg/ml), NBT (0·5 mg/ml) in 0·1 M Tris HCl buffer (pH 8·0).

Biosynthetic pathway of almost all the hormonally active steroids involves the conversion of Δ^5 3β-hydroxysteroids into their ketoforms and the enzyme system carrying out this reaction has been called Δ^5 3β-hydroxysteroid dehydrogenase which preferentially utilises NAD as the coenzyme[6,7]. In the histochemical reaction, if this enzyme is present in the tissue, it oxidises the steroid substrate and the hydrogen is finally accepted by tetrazolium salt which gets reduced to the coloured, insoluble formazan formed at the site of reaction in the tissue section. $NADH_2$-tetrazolium reductase or

NADH$_2$-diaphorase catalyses the latter part of the reaction. Hence the presence and distribution of NADH$_2$-diaphorase in the tissue is one of the limitations in the histochemical localization of Δ^5 3β HSDH, and it should either be ubiquitous or at least be present at the site of Δ^5 3β-HSDH[3]. The possible explanations for the formazan observed in the seminiferous tubules in *M. carinata* are the following: (i) a false positive reaction or "nothing dehydrogenase" effect which may be pH dependent, (ii) non-specific reduction of the tetrazolium, probably non-enzymatic, (iii) a broad-spectrum alcohol dehydrogenase in the seminiferous tubules capable of oxidising the steroid substrate[8], (iv) differential histochemical threshold of the localisation of Δ^5 3β-HSDH in the seminiferous tubules and the interstitial cells of the testis, selectively demonstrable at different values of pH[2].



FIGS. 1–2. Fig. 1. Cross-section of the testis of *M. carinata* showing Δ^5 3β-HSDH activity in the interstitial cells at pH 8·0 (8 × 40). Fig. 2. Cross-section of the testis showing the formazan mainly in the seminiferous tubules at pH 7·5 (8 × 10).

Δ^5 3β-HSDH which has been demonstrated in the interstitial cells of many reptiles[9–12], and *in vitro* biochemical conversions[13,14] strongly point out, the involvement of Leydig cells in the interstitium of the reptilian testis in the biosynthesis of androgens. The demonstration of this enzyme, in interstitial cells of the testis in *M. carinata*, hardly leaves any doubt as to their potentiality in steroidogenesis, though involvement of seminiferous tubules at least partly cannot be ruled out.

The authors wish to thank Prof. M. R. Rajasekharasetty for his encouragement and C.S.I.R. (India) for awarding a Research Fellowship to the first author.

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1. Wattenberg, L. W., *J. Histochem. Cytochem.*, 1958, 6, 225.
2. Baillie, A. H., Ferguson, M. M. and Hart, D. Mck., *Developments in Steroid Histochemistry*, Academic Press, N.Y., 1966.
3. Shivanandappa, T. and Sarkar, H. B. D., *Curr. Sci.*, 1976, **45**, 834.
4. — and —, *Herpatologica*, 1977 (In press).
5. — and —, *Symp. Gen. Comp. Endocrinol.*, Delhi, 1976.
6. Dorfman, R. F. and Ungar, I., *Metabolism of Steroid Hormones*, Academic Press, N.Y., 1965.
7. Samuels, L. T., Helmreisch, M. L., Lasater, M. B. and Reich, H., *Science*, 1951, **113**, 490.
8. Levy, H., Deane, H. W. and Rubin, B. L., *Endocrinology*, 1959, 65, 932.
9. Nandi, J., *Amer. Zool.*, 1967, **7**, 115.
10. Gouder, B. Y. M. and Nadkarni, V. B., *Curr. Sci.*, 1976, **45**, 102.
11. — and —, *Indian J. Exp. Biol.*, 1974, **12**, 326.
12. Erpino, M. J., *Gen. Comp. Endocrinol.*, 1971, **17**, 563.
13. Tam, W. H., Phillips, J. G. and Lofts, B., *Ibid.*, 1969, **13**, 117.
14. Chieffi, G., *Amer. Zool.*, 1972, **12**, 207.

INDUCED PARASITISM OF A FREE-LIVING NEMATODE, *ACROBELOIDES APICULATUS* (THORNE, 1925) IN DIPTEROUS LARVAE

STEINER AND BUHRER[2] and recently Wasilewska and Webster[4] have reviewed the importance of free-living nematodes as disease factors of man and his crops. *Acrobeloides apiculatus* (Thorne, 1925) Thorne, 1937, a free-living nematode is of frequent occurrence in Aligarh soils. The latter also usually contains dipterous larvae of an unidentified spcies. The nematodes were raised *in vitro* on Nigon's agar medium (bacteriological). Although in nature the nematodes were never found to parasitise the dipterous larvae, but when introduced artificially by making a small incision at the anterior end of larvae they started to live in the coelom as parasites without themselves showing any obvious signs of discomfort. When gravid females of *A. apiculatus* from the culture were introduced within the body cavity of dipterous larvae (Fig. 1) they failed to lay eggs which then began to develop inside the uterus (intra-uterine egg development). The hatching also took place within the uterus and the juveniles of *A. apiculatus* began to feed upon the body tissues of the mother which ultimately died and the juveniles were liberated in the coelom of the dipterous larvae.

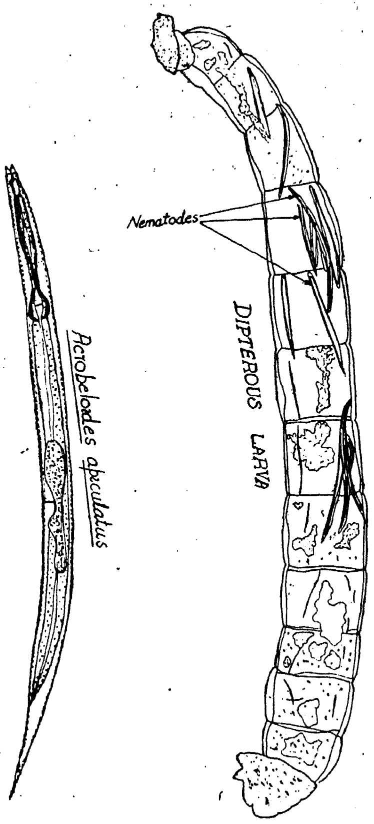

Fig. 1. Showing the nematode, *Acrobeloides apiculatus* and the dipterous larva with the nematodes within its body cavity.

They moved freely within the body cavity of the insect which started showing signs of sluggishness. The growing juveniles now began to feed upon the body contents of the dipterous larva which resulted in its ultimate death. The nematodes did not leave the dipterous larva but continued to feed upon its dead tissue and grew to maturity. The cuticle of the insect larva which was used by the nematodes as a protective cover ultimately gave way setting them free. This appears to be an interesting and unique case of induced parasitism. Another species of this genus, *A. buetschilii* was reported by Thorne[3] to feed on the remnants of the internal organs of *Heterodera schachtii*, the sugar-beet cyst eelworm.

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1. Nigon, V., *Annls Sci. Nat.*, 1949, 2, 1.
2. Steiner, G. and Buhrer, Edna M. *Plant Dis. Reptr.*, 1944, **17**, 172.
3. Thorne, G., *Proc. helminth. Soc. Wash.*, 1937, **4**, 1.
4. Wasilewska, L. and Webster, J. M., *Intern. J. Environmental Studies*, 1975, **7**, 201.

INFLUENCE OF METHOD OF INOCULATION ON SPORULATION OF SOME LIGHT-REQUIRING FUNGI

It has been reported earlier from this laboratory[1] that an isolate of *Phoma* and *Ascochyta pisi* (which normally require) light for sporulations sporulate even in darkness when grown on cellophane film or filter-paper overlying agar medium. Further work on these two fungi and a few others has revealed that the method of inoculation of agar plates also influences sporulation considerably. The results of these experiments are presented here.

Besides the *Phoma* isolate (Ph 1) and *Ascochyta pisi* (AP 1) used in the earlier experiments[1], a second isolate of *Phoma* (Ph 2) and one of *Leptosphaerulina arachidicola* (LA) isolated from groundnut leaves and an isolate of *A. pisi* (AP 2) originally procured from Baarn were used in the present studies. Isolates Ph 1 and 2, AP 1 and LA sporulate normally only when exposed to light from fluorescent daylight or black light lamps. AP 2 did not form pycnidia even when exposed to light. Czapek's agar medium containing 0·1% yeast extract with or without cellophane film overlying the agar was inoculated by two different methods. Either a bit of mycelium from a dark-grown culture was placed in the centre, or a suspension prepared by breaking up dark-grown mycelium with glass beads in sterile distilled water was spread over the surface of the agar or cellophane with a glass spatula. The cultures were incubated in darkness or in light provided by 2 daylight tube lamps (40 W) and 1 Sylvania F 40 BL lamp (30 cm below the lamps) in a 12 : 12 h light-dark cycle. Corning petri dishes were used throughout[2].

The results presented in Table I relate to observations made on the fifth day after inoculation.

When inoculated at one point (centre of plate) Ph 1 and AP 1 behaved as before[1] sporulating even in darkness when cellophane was present. Ph 2 needed presence of cellophane even in light for sporulation. AP 2 did not sporulate with this method of inoculation under any condition. LA formed only sterile perithecia except in cultures raised under light on cellophane. However, the results were dramatically different when mycelial suspensions of these fungi were spread over the agar/cellophane. When inoculated in this manner, Ph 1 and AP 1 sporulated in dark even in the absence of cellophane. Ph 2 no longer required cellophane for pycnidial production in light. AP 2 formed sterile pycnidial bodies in light as well as darkness on cellophane. Fertile and produced sterile flowers, the flowers were smaller in size and remained closed till shedding.

Its flower buds were examined cytologically. The PMCs contained only thirteen chromosomes ($n = x = 13$) (Fig. 2). PMCs with bivalents were also observed (Fig. 3). The number of bivalents ranged from 0–6. The pollen grains were highly variable in size and did not stain with 1% IKI solution. The plant being sterile is vegetatively propagated for further studies.

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Rahuri, *February* 4, 1977.

1. Barrow, J. R. and Chaudhari, H. K., *Crop. Sci.*, 1976, **16**, 441.
2. Kimber, G. and Riley, R., *Bot. Rev.*, 1963, **29**, 480.
3. Skovsted, A., *Jour. Genet.*, 1935, **30**, 447.
4. Turecotte, E. L. and Feaster, C. V., *Crop. Sci.*, 1969, **9**, 653.
5. Webber, J. M., *Bot. Rev.*, 1940, 6, 575.

SPODOPTERA LITURA FAB. CAUSING DAMAGE TO RAMIE IN ASSAM

THE leaf-eating caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera), has been noted for the first time to cause considerable damage to ramie (*Boehmeria nivea* Gaud.), which yields the strongest known vegetable fibre[2], at the Ramie Research Station, Sorbhog, Kamrup, Assam. *Spodoptera litura* is a polyphagous pest of agricultural importance throughout India and has been reported to occur on various crops including cotton, okra, castor, tobacco, jute, tomato, cowpea, banana, red gram, cabbage[1,4–6]. In the South-East region of Rajasthan, it appears to be one of the major biological factors responsible for the damage of *Kharif* crops[3].

The pest attacks ramie at all stages of growth and becomes prevalent throughout monsoon season. The caterpillars, in their early stage, feed gregariously on the parenchyma tissues of the leaves; and as they grow, they scatter over the entire field and eat the leaf blades and other tender parts showing the cutworm habit. Sometimes, the grown-up larvae defoliate the plants.

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1. Das, G. M., *Sci. and Cult.*, 1948, **14**, 186.
2. Ghosh, K., *Indian Farmer's Digest*, 1969, **2**, 11, 33.
3. Pandey, S. N. and Srivastava, R. P., *Indian J. Ent.*, 1967, **29**, 229.
4. Rattan Lal and Nayak, G. N., *Ibid.*, 1963, **25**, 299.
5. Thobbi, B. V., *Ibid.*, 1961, **23**, 262.
6. Thomas, M. J., Abraham, J. and Nair, M. R. G. K, *Indian J. Agric. Sci.*, 1969, **39**, 5, 400.

GLANDLESS HAPLOIDS $n = 2x = 26$ IN *GOSSYPIUM HIRSUTUM*, L. (COTTON)

SIX haploids ($n = 2x = 26$) were obtained in the X_2 progeny of the 25 kr. irradiated mutant in *Gossypium hirsutum*, L. cotton, variety High Gossypol (H.G. 108). The plants were highly self-sterile but fairly cross-fertile. The recessive condition gl_1, gl_2 and gl_3 has been confirmed under haploid level in *G. hirsutum* cotton.

Hutchinson *et al.* (1947)[2] considered oil glands as an important taxonomic character useful in classification of cotton and its relatives. Kimber and Riley (1963)[3] reviewed literature on haploid angiosperms including *G. barbadense* cottons, and Skovsted (1935)[6] and Webber (1940)[9] reported the occurrence of monoploids with diploids ($2n = 2x = 26$) in *G. davidsonii* Kell. and *G. sturtii*, Muell. Haploids in the interspecific hybrids by semigamy is also reported by Turecotte and Feaster (1969)[8] and Barrow and Chaudhari (1976)[1]. The authors (in press)[7] have also recorded a monoploid in *G. arboreum* var. LD. 132.

X-ray treatment at 25 kr dose in H.G. 108 induced one completely glandless plant (H.G. 108–25–1) in September, 1975, which was normal in morphology and fertility. Meiotic studies of this plant did not indicate any chromosomal variations. Six glandless plants with retarded growth, smaller leaves and short internodes and with sterile flowers were noticed in March, 1976 in the selfed (X_2) progeny of the above glandless mutant. PMCs studied in young flower buds of all the six plants revealed the presence of $n = 2x = 26$ chromosomes with an average of six bivalents (Fig. 2). Meiosis was highly irregular. At anaphase I and II there was unequal separation ranging from ten to sixteen chromosomes at each pole. Only tetrads were observed at the end of second meiotic division. Pollen grains when tested with 1% IKI solution showed 90–96% sterility. The plant were highly self-sterile but fairly cross-fertile. Fev bolls were set on haploids (Fig. 3) when crosse with normal tetraploid glandless mutant and als few seeds were obtained from its crosses with culti vated diploids, *i.e.*, *G. arboreum*, *G. herbaceum* an wild diploids *G. thurberi*, Tod. and *G. anomalum* Wa and Peyr. Few bolls were set with one or two seeds after selfing, might be due to parthenocarpy.

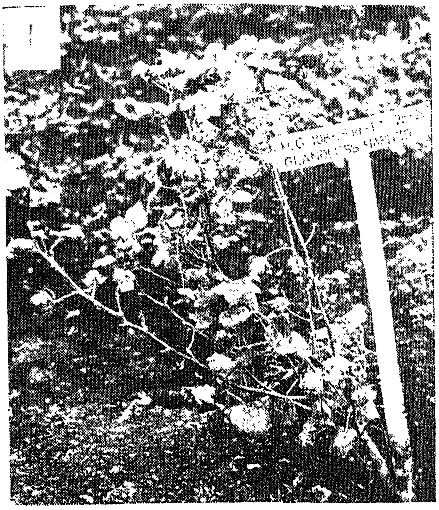

FIG. 1. A glandless haploid ($2n = 26$) in X_2 of *G. hirsutum* cotton.

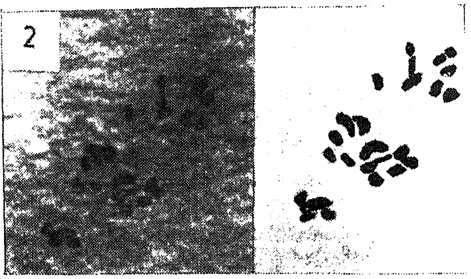

FIG. 2. Metaphase-I showing 6 II + 14 I 950 ×.

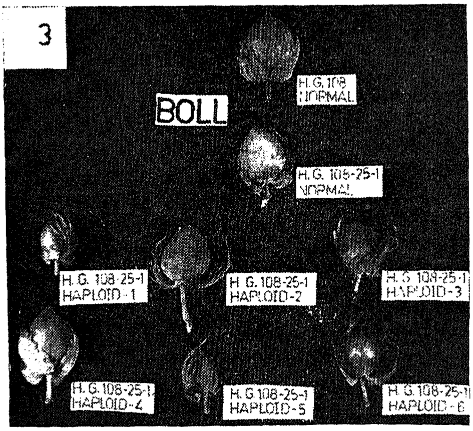

FIG. 3. Mature bolls of glandless haploid ($2n = 26$) and H.G. 108 Normal glanded *G. hirsutum* ($2n = 52$).

The recessive glandlessness of the plant conditioned by gl_1, gl_2 and gl_3 (McMichael, 1954 and 1960)[4,9] which is induced in tetraploid (X_1 generation) is confirmed at haploid level in X_2 generation. The haploids are being treated with colchicine to induce true breeding glandless lines, and this will be an additional source of variability for gossypol free oil and protein content in *G. hirsutum* cotton. This work is in progress.

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1. Barrow, J. R. and Chaudhari, H. K., *Crop. Sci.*, 1976, **16**, 441.
2. Hutchihson, J. B., Silow, R. A. and Stephens, S. G., *The Evolution of* Gossypium. Oxford University Press, 1947.
3. Kimber, G. and Riley, R., *Bot. Rev.*, 1963, **29**, 480.
4. McMichael, S. C., *Agron. J.*, 1954, **46**, 527.
5. —, *Ibid.*, 1960, **52**, 386.
6. Skovstead, A., *Jour. Genet.*, 1935, **30**, 447.
7. Thombre, M. V. and Mehetre, S. S. (1977), under publication.
8. Turecotte, E. L. and Feaster, C. V., *Crop. Sci.* 1969, **9**, 653.
9. Webber, J. M., *Bot. Rev.*, 1940, **6**, 575.

RADIATION INDUCED VARIEGATED MUTANT IN *BOUGAINVILLEA*

HOLTTUM[1] described four species of cultivated *Bougainvillea*, namely *B. spectabilis* Willd., *B. glabra* Choisy, *B. peruviana* Humb. and Bonpland, and *B.* × *buttiana* Holtt. and Stand. The group *B.* × *buttiana* is believed to be a natural interspecific hybrid complex between *B. peruviana* and *B. glabra*. *B.* cv. 'Jayalakshmi' has fuchsia-purple bracts and it was raised through seedlings from cultivar 'Meera' of *B.* × *butitana* hybrid complex. Thus 'Jayalakshmi' is one among the 20 cultivars, originated and isolated through seedlings from the different centres of the world[2]. In the course of our investigations on inducing mutations in ornamental plants, a mutant with leaf variegation obtained from cv. Jayalakshmi, following chronic gamma ray treatment, is reported here.

In September, 1973 potted cuttings of *Bougainvillea* were exposed to chronic gamma-ray doses, from a ^{60}Co field source with the dose rates ranging from 10–30 R/h, for a total of 20 days. Of the 33 'Jayalakshmi' plants which received the accumulated dose of about 2·5 kR, 26 survived in the following season. They were further exposed to a total dose of about 4 kR at a dose rate of 22 R/h during January, 1975.

Bougainvillea in general is sensitive to low doses of radiations and with acute doses of X-rays and gamma-rays, it cannot withstand doses beyond 2 kR administered to fresh cuttings. With chronic gamma-ray doses, there was no survival beyond 5 kR. It was therefore found suitable to administer recurring low doses of radiations to increase surviving populations and to induce mutations. During these studies, in one plant, one of the branches originated from the irradiated shoot which appeared as normal, produced four sprouts with variegated leaves, as sectorial chimeras, during June, 1975. These four branches were later multiplied vegetatively and established as a new cultivar named 'Jayalakshmi variegata', the salient features of which is given here:

Bouganinvillea × *buttiana* Holttum and Standley cv. *Jayalakshmi variegata* Abraham and Desai.

Young leaves coppery red; mature leaves with cream yellow variegations on the margins and lamina, with a green patch at the centre. Bracts brick red while young, turning to fuchsia-purple when mature. Floral tubes purple as in cv. Jayalakshmi and thus resembling the control except for leaf variegation (Fig. 1).

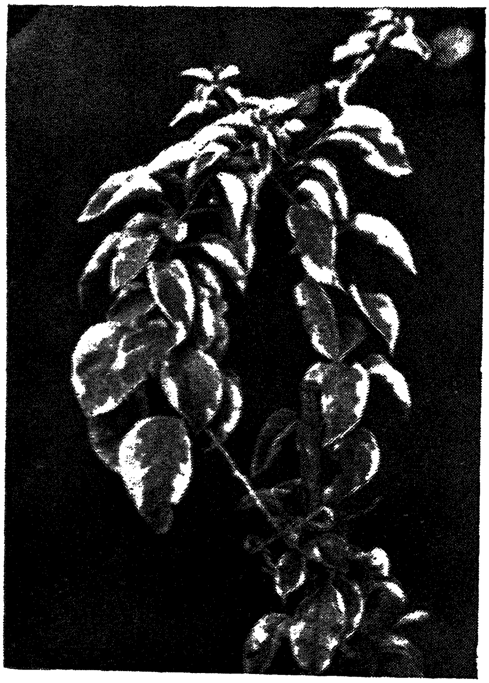

FIG. 1. *Bougainvillea* mutant '*Jayalakshmi variegata*'.

Leaves n T.S. of the control showed one lyaer of palisade on the adexial side and the 2–8 layered mesophyll filled with chlorophyll, except the upper and lower epidermal layers. The variegated leaves of the mutant also showed a single layer of palisade but chlorophyll was present only in the inner mesophyll ells of the green area. The variegated leaves appeared more light green on the lower side. The growth of the variegated mutant is less vigorous and is late flowering compared to control, probably because of the reduced chlorophyll content in the leaves.

In *Bougainvillea*, even though there are about twenty variegated cultivars reported so far, invariably they were all isolated as bud spots at different centres, except the one isolated in cv. 'Partha' by gamma radiation[3]. The mutant 'Jayalakshmi variegata' is of better ornamental value even during vegetative periods.

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April 15, 1977.

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1. Holttum, R. E., *Malay Agri. Hort. Ass. Mag.*, 1955, **12** (3), 2.
2. Pal, B. P. and Vishnu Swarup, *Bougainvilleas*, Indian Council of Agri. Res., New Delhi, 1974.
3. Gupta, M. N. and Shukla, R., "Mutation breeding of tuberose, *Polyanthes tuberosa* L.," *Proc. Symp. Use of Radiations and Radioisotopes in Studies of Plant Productivty*, Department of Atomic Energy, G. B. Pant University of Agri. and Tech., Pantnagar, 1974, p. 169.

DACTYLARIA INDICA AGARWAL AND CHOWDHRY, A NEW FUNGUS FROM SOIL

IN continuation of our studies on *Hyphomycetes* a species of the genus *Dactylaria* was isolated by the authors from the garden soil. Earlier Saccardo[12] described a number of species from decaying wood and leaves including *Dactylaria purpurella* as a type Drechsler[2-5], Duddington[6,7], Peach[9], Juniper[8], Shome and Shome[13] have described a number of *Dactylaria* species as parasites on free living terricolous nematodes. Routein[10] described *D. lutea* from Argentina soil while Roy and Gujarati[11] have described *D. fulva* from soil in India.

Taxonomically *Dactylaria* is close to *Dactylella* and *Diplorhinotrichum*. Bhatt and Kendrik[1] studying these two genera placed eleven species of *Diplorhinotrichum* in synonymy with *Dactylaria*. They also erected a new species *D. humicola* from soil of Canada. The present isolate does not match with any of the described species of *Dactylaria* with regard to the characteristic shape, size, septation of conidiophores and conidia. Hence a new species *Dactylaria indica* is being proposed. The culture has been deposited at Indian type culture collection, I.A.R.I., New Delhi, under Accession No. 2228.

Dactylaria indica sp. nov. (Figs. 1–3)

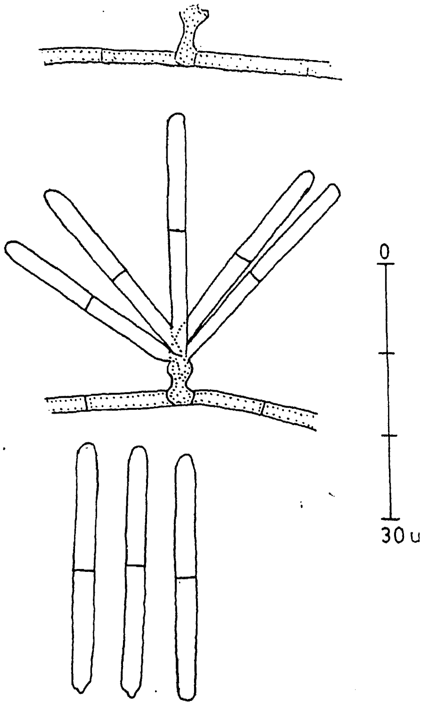
Colonies on Potato Dextrose Agar medium 3·5 cm diam. after 15 days at 25° C, at first white or cream coloured, reverse brown, at maturity becoming grey; aerial mycelium forming fascicles, yellowish brown at maturity; hyphae hyaline, septate, thin walled, sparingly branched, 1–2 μ wide; Conidiophores, measuring 5–8 × 2–3 μ, arise laterally as a round structure or a branch from the hyphae, the terminal sympodula swollen at its apex; 2–6 conidia produced at denticle apices of the conidiophore, hyaline, cylindrical, 2-celled, thin walled, measuring 15–30 × 2–3 μ with a flat obstriction scar at the base.

Habitat: From soil, India.

Fig. 1

Fig. 2

Fig. 3



Figs. 1–3. Dactylaria indica
1. Conidiophore
2. Conidiophore and conidia
3. Conidia

Latin diagnosis

Coloniae in agaro solano-dextroso 3·5 cm in aetate, 15 dierum ad grad 25° C, primo albae vel eburneae. facie versa brunnea, ad maturation evadentes griseae, Mycelium aerium fasciculos formans, luteolos, brunneos ad maturitatum. Hyphae hyalinae, septate, parietibus tinuis, parse ramosus, 1–2 μ lat. Conidiophora 5–8 × 2–3 μ lateraliter emergentia ut structura rotunda vel ut ranus e hypha, cellula apicalis (sympodula) at apicem. 2–6 conidia ad apices dentatos conidiophorum, hyalina, cylindrica, 2-cellularia, parietibus tenuis, 15–30 × 2–3 μ, cicataricem basalem conspicuam exhibentia.

Habitat: in solo, Indiae.

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December 20, 1976.

1. Bhatt, G. C. and Kendrik, W. N., *Can. J. Bot.*, 1968, **46**, 1253.
2. Drechsler, C., *Mycologia*, 1937, **19**, 447.
3. —, *Ibid.*, 1944, **36**, 138.
4. —, *Ibid.*, 1950, **42**, 1.
5. —, *Sydowia*, 1962, **15**, 9.
6. Duddington, C. L., *Trans. Brit. mycol. Soc.*, 1951, **34**, 194.
7. —, *Ibid.*, 1951, **34**, 322.
8. Juniper, A. J., *Ibid.*, 1954, **37**, 437.
9. Peach, M., *Ibid.*, 1952, **35**, 19.
10. Routien, J. B., *Mycologia*, 1957, **49**, 188.
11. Roy, R. Y. and Gujarati, S., *Lloydia*, 1965, **28**, 53.
12. Saccardo, P. A., *Sylloge Fungorum*, 1886, **4**, 194.
13. Shome, S. K. and Shome, U., *Mycopath. Myco. appl.*, 1966, **30**, 216.

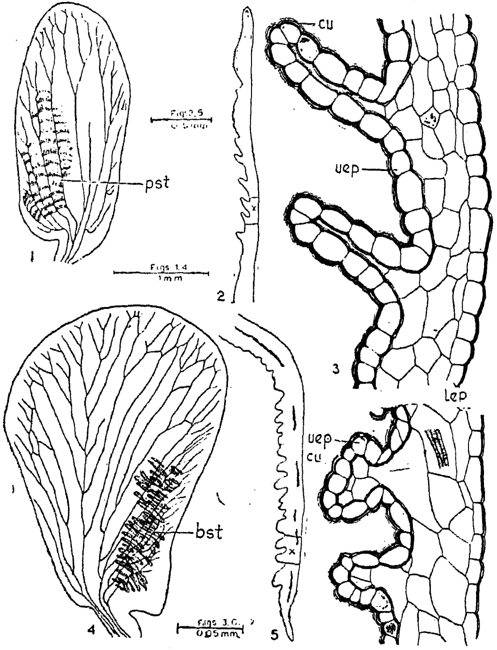
SOME INTERESTING FEATURES IN THE FLOWER OF *CROTALARIA* AND *TEPHROSIA* SPECIES

DURING our studies on the floral morphology of four species, *viz.*, *Crotalaria burhia* Buch.-Ham., *C. medicaginea* Lamk., *Tephrosia hamiltonii* Drumm. and *T. apollinea* Link some interesting features on the 'wing' petals were observed.

The petals were cleaned in lactic acid and stained in basic fuchsin. In *Crotalaria burhia* and *C. medicaginea* the two lateral wings of the flower have pocket-like structures which are located on the outer surface of lower half region. They are more in number towards the proximal end. These pockets have their open ends directed downwards, *i.e.*, towards the pedicel of the flower. They are deep enough to extend up to the mouth of the preceding pocket. These pockets are arranged in regular rows in between the major veins of the petal. The mouth of the pocket is lined by epidermal epithelium (Fig. 1). However, in *Tephrosia*, both the species have band-like structures instead of pockets. These are irregularly distributed and spread over the veins also (Fig. 4). They are not so deep as in *Crotalaria* species. But they occupy the same position on the wing petals as the pockets in *Crotalaria* species.

Anatomically, a longitudinal section of the wing petal in both *Crotalaria* species shows an upper and a lower epidermis lined by a thin cuticle and enclosing a parenchymatous mesophyll (Fig. 3). The cells of upper epidermis are small, rectangular and are densely filled with yellowish contents. They have their outer tangential walls more thickened than the inner tangential and lateral walls. The cells of lower epidermis are tangentially elongated with the thickenings along the outer tangential walls only and less pronounced as compared to the outer tangential walls of upper epidermis. The outer wall of the pocket is contributed by the upward extension of the upper epidermis of the wing petal. The cuticle at the apex of the fold is wavy due to some deposition (Figs. 2, 3).

In *Tephrosia* species, in a longitudinal section, these structures appear as ridges. The cells of upper epidermis are small and thick-walled. Outer tangential walls are thicker in comparison with inner tangential walls. The cells of lower epidermis are tangentially elongated with the thickenings on outer tangential walls only. Both the epidermal layers are lined by smooth cuticles. In the region of a band the upper epidermis along with the parenchymatous tissue forms a ridge-like structure (Figs. 5, 6).



FIGS. 1–6. Figs. 1–3. *C. burhia*. Fig. 1. Pocket-shaped structures on the wing petal. Fig. 2 Outline of the same, in l.s. Fig. 3. Portion marked 'X' in Fig. 2, cellular details. Figs. 4–6. *T. hamiltonii*. Fig. 4. Band-like structures on wing petal. Fig. 5. Outline of the same, in l.s. Fig. 6. Portion marked 'X' in Fig. 5, cellular details.

(*bst*, band-like structures; *cu*, cuticle; *lep*, lower epidermis; *pst*, pocket-shaped structures; *uep*, upper epidermis.)

It is difficult to attribute any function to these structures. Faegri and Pijl[1] mentioned some folds on the wing petals of Leguminosae, while discussing the pollination ecology of the family. They described that these structures are meant for connation between wing and keel petals. However, no connation is observed in these petals and since they are present along the outer face of the wing petals only, such a connation is not possible.

I am extremely grateful to Dr. N. Chandra for guidance, Prof. B. Tiagi for encouragement and Dr. C. M. Govil for helpful suggestions.

Dept. of Botany, (MISS) ARVINDER KAUR NARANG.
University of Rajasthan,
Jaipur, *August* 27, 1976.

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1. Faegri, K. and Pijil, L. V., *The Principles of Pollination Ecology*, II ed., Pergamon Press, Oxford, 1971.

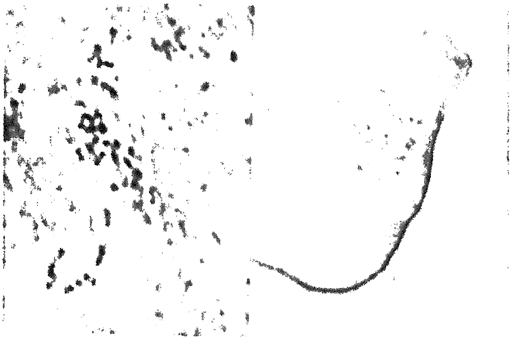
PARTHENIN—A PRETREATMENT REAGENT FOR CHROMOSOMES

Parthenium hysterophorus L., a troublesome weed in several parts of India, is found to contain growth toxins. Parthenin, which is the principal component of the inhibitors, constitutes 0·32% by dry weight of the leaves and causes inhibition of linear growth in seedlings of wheat and other crop plants. Hence, a study of the effects of this sesquiterpene lactone on different aspects of cell division in relation to inhibition of root growth was made. It was also examined whether this toxic principle could serve as a pretreatment reagent in the preparation of root tip squashes for karyotype analysis.

Parthenin was obtained in crystalline form from the aqueous leachate of the aerial parts of the weed following the method of Sukhada[1], with slight modification discussed later. Root tips of *Zephyranthes rosea* Herb., *Chlorophytum elatum* R. Brown and *Parthenium hysterophorus* L., 2 mm long were excised from healthy plants, washed with tap water followed by distilled water and treated with 0·5% solution of parthenin for 1 hr. Controls were treated similarly with distilled water. Roots from onion bulbs, growing actively in water, were also treated with the test solution for 1 hr, 24 hr and 48 hr. After the treatment, the root tips were fixed in Carnoy's fluid at room temperature. Using root tips of 1 mm length, 20 aceto-orcein squashes were prepared for each treatment. In each, the number of metaphase plates in 200 cells was scored in the region showing maximum cell division. Percentage of cells showing abnormalities was recorded, as also the qualitative nature of the affected cells.

In all the four species tested, 1 hr treatment caused mitotic arrest resulting in high frequency of metaphase plates. Chromosomes were well spread and condensed compared to those in preparations following distilled water treatment (Figs, 1, 2 and 3). In onion, 24 hr and 48 hr treatment caused cessation of root growth and several abnormalities like anaphase inhibition, chromosomal laggards, stickiness, vacuolisation (Fig. 4), irregularly shaped nuclei and binucleate cells. The treatment for 24 hr caused abnormality in 46% of the cells and with 48 hr treatment it increased to 85%.

Thus, pretreatment of the roots with 0·05% aqueous solution of Parthenin for 1 hr is beneficial in



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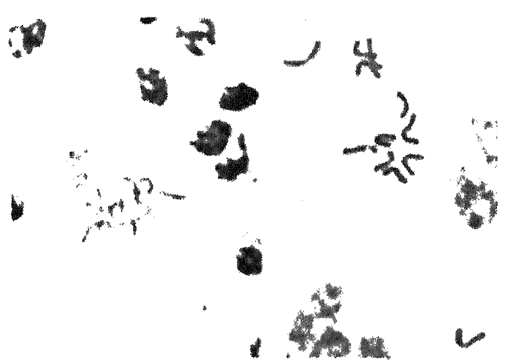

[illegible] *Parthenium* [illegible] parthenin [illegible]

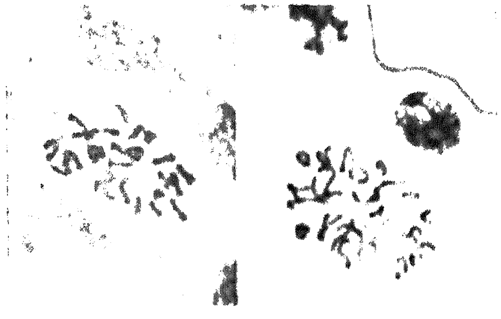

FIG. 3. [illegible] *Allium cepa* [illegible] treated with parthenin [illegible] condensation of chromosomes. [illegible]

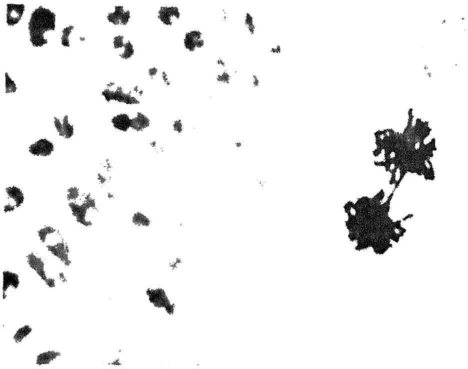

FIG. 4. Onion root tip cells pretreated with 0.05% [illegible] showing chromosomal [illegible] condensed chromosomes and increased vacuolation in the nuclei.

[illegible] condensation of the chromosomes [illegible] metaphase plates [illegible] comparable [illegible] Parthenin [illegible] as high as 0.5% [illegible] considerably higher if extracted [illegible] water [illegible] present investigation [illegible] with [illegible] water as reported in the original procedure[1]. *Parthenium hysterophorus* can be exploited commercially for the production of parthenin in view of its high concentration and the ease with which it can be extracted.

Table showing the increased number of metaphase plates due to parthenin treatment

Species tested	Number of cells showing metaphase plates in 200 cells*	
	Distilled water	Parthenin 0.05%**
Chlorophytum elatum	[illegible]	8
Zephyranthes rosea	4	9
Parthenium hysterophorus	9	15
Allium cepa	4	9

* Average of 20 preparations, ** Significant at 5% level.

Senior author thankfully acknowledges Dr. Jayachandra for encouragement and Dr. Seetharam and Mr. D. S. Krishnamurthy for their help in photography. Our thanks are also due to Professor M. Nagaraj for the facilities provided.

Department of Botany, SUKHADA D. KANCHAN.
Bangalore University, K. S. GEETHA.
Bangalore, *March* 21, 1977.

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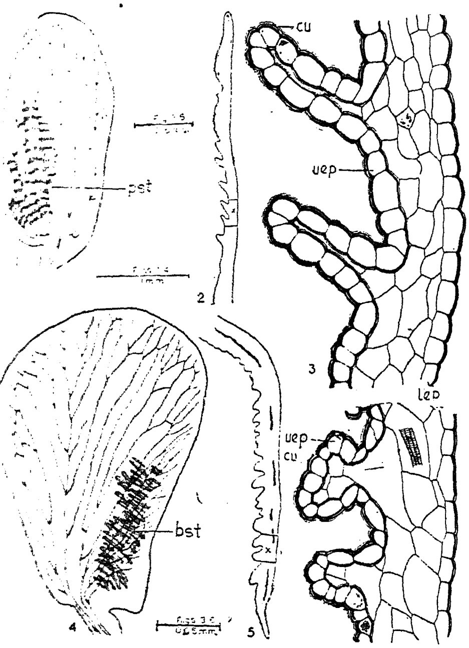
1. Sukhada D. Kanchan, *Curr. Sci.*, 1975, **44** (10), 358.

RADICULAR PROLIFERATION IN THE SEEDLINGS OF *PHASEOLUS RADIATUS* LINN. BY COUMARIN

The role of coumarin in germination inhibition has already been envisaged (Sigmund, 1914). Mayer and Evenari[2] (1952) studied the relation between the structure of coumarin and its derivatives and their activity as germination inhibitors in monograph by Mayer

In *Tephrosia* species, in a longitudinal section, these structures appear as ridges. The cells of upper epidermis are small and thick-walled. Outer tangential walls are thicker in comparison with inner tangential walls. The cells of lower epidermis are tangentially elongated with the thickenings on outer tangential walls only. Both the epidermal layers are lined by smooth cuticles. In the region of a band the upper epidermis along with the parenchymatous tissue forms a ridge-like structure (Figs. 5, 6).



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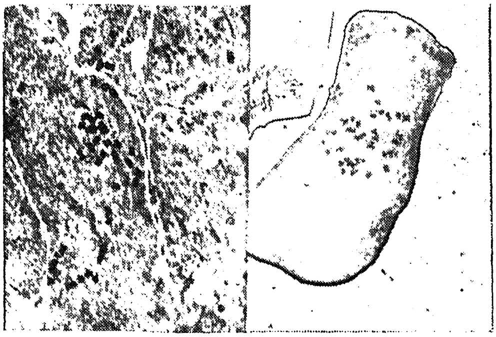

FIG. 1. Root tip cells of *B. hysterophorus* ($2n = 32$) pretreated for 1 hr with 0·05% parthenin showing better condensation of chromosomes. Control on the left.

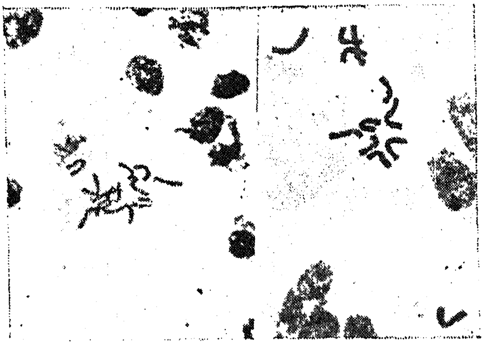

FIG. 2. Root tip cells of *Zephyranthes rosea* ($2n = 12$) pretreated for 1 hr with 0·05% parthenin showing better condensation of chromosomes. Control on the left.

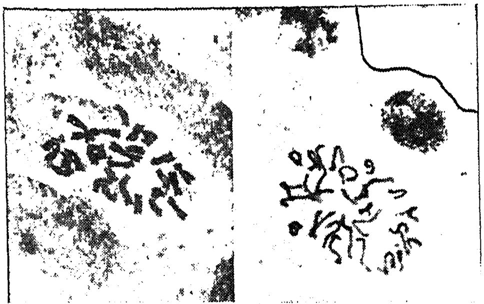

FIG. 3. Root tip cells of *Cholorophytum elatum* ($2n = 28$) pretreated for 1 hr with parthenin showing better condensation of chromosomes. Control on the right.

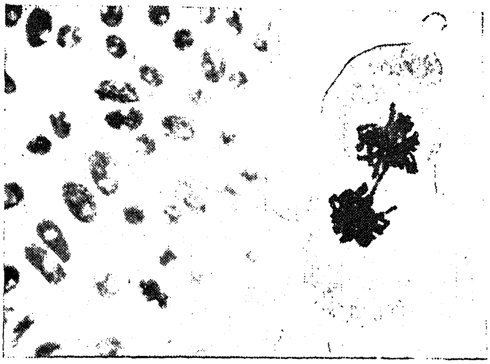

FIG. 4. Onion root tip cells pretreated with 0·05% parthenin for 48 hr showing chromosomal laggard (right), stickiness of chromosomes and increased vacuosation in the nuclei.

realisation of proper condensation of the chromosomes and high frequency of metaphase plates for karyotype analysis, the effect of the chemical being comparable to that of colchicine and hydroxyquinoline. Parthenin content in leaves and inflorescence is as high as 0·32%. Yield of parthenin will be considerably higher if extracted with either hot water or 80% methanol (as followed in the present investigation) than with cold water as reported in the original procedure[1]. *Parthenium hysterophorus* can be exploited commercially for the production of parthenin in view of its high concentration and the ease with which it can be extracted.

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SUKHADA D. KANCHAN.
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RADICULAR PROLIFERATION IN THE SEEDLINGS OF *PHASEOLUS RADIATUS* LINN. BY COUMARIN

THE role of coumarin in germination inhibition has already been envisaged (Sigmund, 1914[5]). Mayer and Evenari[3] (1952) studied the relation between the structure of coumarin and its derivatives and their activity as germination inhibitors monograph by Mayer and Poljakoff-mayber, 1963[4]). The inhibitory action of this compound on cotyledonary chlorophyll synthesis has been examined by Knypl (1967,[1] 1970[2]). Coumarin has been shown to inhibit the synthesis of chlorophyll in the leaf tissues of etiolated maize seedlings (Knypl, 1970[2]), Svensson (1971[6], 1972a[7], 1972 b[8]) made an extensive examination of the effect of coumarin on the growth, histology and dry matter, protein and nucleic acid contents of roots of wheat and maize. Coumarin has been envisaged to show an important morphogenetic effect on root through destruction or disarrangement of a formative system (Svensson, 1972 c[9]).

The seeds were washed in distilled water, surface-sterilized in 0·1% $HgCl_2$ and kept in petridishes with distilled water-moistened filter-paper. Treatment with 20 PPM coumarin was carried out on germinated seeds 24 hr after soaking.

During our investigations on post-germinational growth rates of *Phaseolus radiatus*, seedlings with abnormal numerical proliferation of radicles were noted (Figs. 1 and 2) in a treatment with 20 PPM of coumarin. The incidence of this abnormolity was not more, but as compared to the seedlings treated with distilled water (controls), this was important.

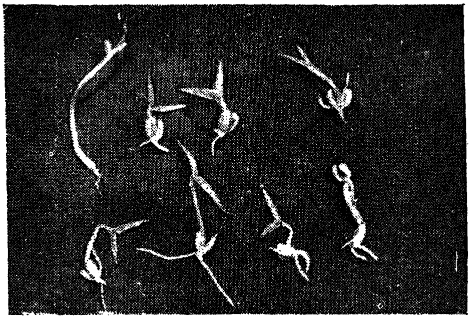

FIG. 1. Radicular proliferation in the seedlings of *Phaseolus radiatus*. The photographs were taken on the 2nd day after treatment with the chemical.
No. 1, seedling distilled water treated control; Nos. 2 to 8, coumarin treated seedlings. Radicular duplication (2, 3), triplication (4, 5, 6, 7) and quadruplication (8) are evident. The remarkable absence of hypocotylar portion may be noted.

The abnormality was besides the general suppression of elongation of radicle and proliferation of lateral roots (H. R. Gopala Krishna, unpublished observations). One significant aspect noted in conjunction with the present abnormality was a remarkable suppression of growth of hypocotyl.

Svensson (1971[6] and 1972[7-9]) suggested that the random reorientation of the mitotic spindle is an important cellular event brought about by coumarin in the growing root. The proliferation of lateral roots is probably caused by such a cellular event. The proliferation of radicles noticed in the present work appears to be rather different from the effect of coumarin noticed by Svensson in the monocots of maize and wheat. This appears to result from an abnormal splitting of the meristem itself. Further work is in progress.

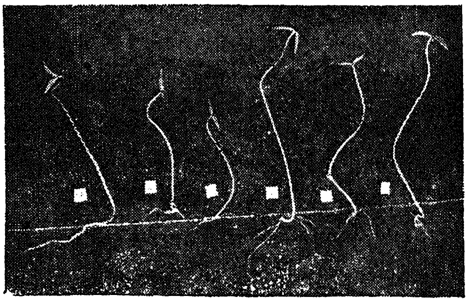

FIG. 2. The photographs were taken on the 4th day after the treatment with the chemical.
No. 1, distilled water treated control, Nos. 2, 3, 4, 5, 6—coumarin treated seedlings. Radicular proliferation is evident in all the treatments.

The authors express their deep sense of gratitude to Mr. S. Nagaiah, Principal and Mr. V. Chandrasekharam and other colleagues of the Botany Department and the Management of Tirupati Tirumala Devasthanams for encouragement.

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Department of Botany,
Sri Venkateswara University,
Tirupati 517 502.
February 28, 1977.

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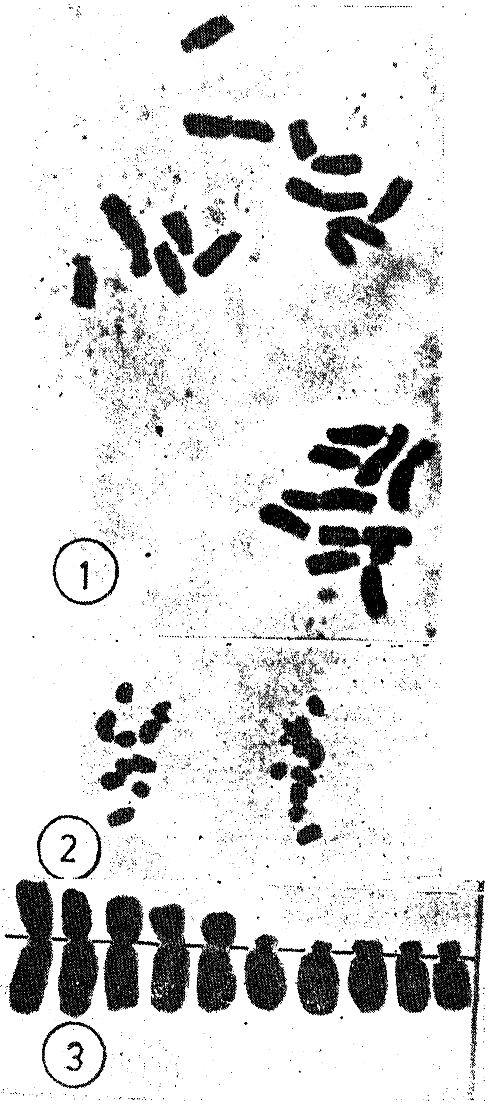
1. Knypl, J. S., *Planta* (Berl.), 1967, **72**, 292.
2. —, *Biochem. Physiol. Pflanzen, Bot.*, 1970, **161**, 8, 1.
3. Mayer, A. M. and Evenari, M., *J. Exptl. Bot.* 1952, **8**, 246.
4. — and Poljakoff-mayber, *The Germination o Seed*, Internat. ser. Monogr. Pure and Appl. Biol. Divn. Plant. Vol. 3, Pergamon Press, Oxford, 1963.
5. Sigmund, W., *Biochem. Zeit.*, 1914, **62**, 220.
6. Svensson, S. B., *Physiol. Plant.*, 1971, **24**, 446
7. —, *Ibid.*, 1972 *a*, **27**, 23.
8. —, *Ibid*, 1972 *b*, **26**, 115.
9. —, Academical Dissertation, University of Lund, 1972 *c*.

KARYOTYPE OF *TRILOBACHNE COOKEI* (STAPF) SCHNECK *EX* HENRARD

Trilobachene cookei belongs to the tribe Maydeae of Poaceae. It is distributed over limited areas in the Western Ghats of India. The plants are annual herbs growing in pure stands and up to about 2 m in height on the forest slopes. Its striking morphological resemblance to the cultivated maize is the separation of male and female inflorescences in space, *i.e.*, a purely male tassel at the apex and one to three (more commonly two) female flowers, either associated with or without male inflorescence, arising in the leaf axils and enclosed by a common bract.



FIGS. 1–3. Fig. 1. Somatic metaphase from intercalary meristem of leaf sheath showing $2n = 20$ chromosomes. Fig. 2. Anaphase I, showing 10 univalents at each pole. Fig. 3. A 'cut out' genome arranged in decreasing total length.

While some literature exists on the oriental genera of Maydeae like *Coix* and *Chionachne*, even the chromosome number of this genus is unknown. In the present note, chromosome number and a breif account of karyotype of this genus are reported.

T. cookei is no exception to the known genera of Maydeae which comprise genomes consisting of five or ten chromosomes[1] (except *Tripsacum*, $n = 18$). The somatic chromosome counts in this genus reveal $2n = 20$ (Fig. 1), and intercalary meristems of the leaf sheaths show well-defined chromosome morphology compared to root-tip meristems. The chromosome number has been confirmed in the pollen mother cells during meiosis (Fig. 2). The genome, consisting of ten chromosomes, when arranged in decreasing total length (Fig. 3) shows a gradual gradient in size from chromosomes 1 to 5 and 6 to 10, with the last approximately half the first. The karyotype is asymmetrical. Chromosomes are classified on the basis of the position of centromere following Adhikary[2], who modified Levan's method[3] and made finer differences in chromosome grouping based on precise determination of the centromere. On the basis of the ratio of long arm to short arm, four chromosomes are nearly median, three are nearly submedian and three are nearly subterminal. Presence of nearly subterminal chromosomes with high 'r' value in the complement is a rather unique feature of this genus since neither oriental (unpublished data from this laboratory) nor occidental genera[4] of the tribe Maydeae show such chromosomal sets. Details of the karyotype and the behaviour of chromosomes during meiosis are being worked out.

I thank Prof. K. B. Deshpande for facilities, to Miss S. G. Mashalkar for technical help, and to Dr. G. B. Deodikar, Director, M.A.C.S., Poona, for introducing me to this plant material.

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1. Darlington, C. D. and Wylie, A. P., *Chromosome Atlas of Flowering Plants*, George Allen and Unwin Ltd., London, 1955.
2. Adhikary, A. K., *Cytologia*, 1974, **39**, 11.
3. Levan, A., Fredga, K. and Sandberg, A. A., *Hereditas*, 1964, **52**, 201.
4. Longley, A. E., *Bot. Rev.*, 1941, **7**, 263.

OCCURRENCE OF THE BENTHIC GYMNOPLEURAN CRAB *NOTOPUS DORSIPES* (FABRICIUS) IN THE NORTHERN ARABIAN SEA

During the Oceanographic Expedition on I.N.S. Darshak in the Northern Arabian Sea (December 1973 to May 1974) a few male specimens of the Brachyuran crustacean *Notopus dorsipes* (Fabricius, 1798) were collected (Fig. 1) from depths varying from 84 to 100 m at lat. 20° 44′ N and long. 69° 23′ E. Its first record was by Alcock in 1896[1] from the Andamans and off the Southern Malabar Coast and this is the second record in the Indian seas which helps to bridge the gap between the earlier records in the Red Sea[2] and Southern Malabar Coast[1].

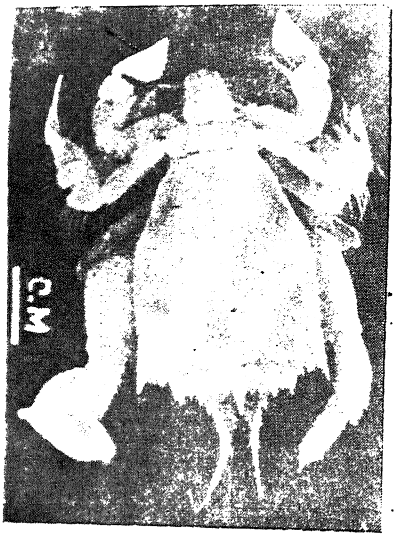

Fig. 1. *Notopus dorsipes* (Fabricius, 1798), dorsal view.

This species has also been reported from off Western Australia[3] and from the Pacific Ocean, *i.e.*, from the East Indies-Siboga Expedition[4] and Japan[5].

The specimens obtained agree well with the general description and figures given by earlier authors, *i.e.*, Alcock, 1896, p. 290 (description); Laurie, 1915, p. 429 (amendment of Alcock's description), Ihle, 1918, p. 294 (Syn. and refs.); Yokoya, 1933[6], p. 112; Sakai, 1937, p. 175, pl, xvi, fig. 1 and 1940[7], p. 40; Holthuis, 1959[8], p. 108, 1962[9], p. 55; Utinomi, 1960[10], p. 73; pl. 37, fig. 2. Tyndale-Biscoe and George, 1962, p. 92 (diagnostic features). The genus *Notopus* de Haan, 1841 of the family Raninidae includes three valid species, *viz.*, *Notopus dorsipes* (Fabricius, 1798), *N. ovalis* Henderson, 1888 and *N. misakiensis* Sakai, 1937. *Notopus ovalis* Henderson has been recorded from Japan and Little ki island and from Australia and *Notopus misakiensis* Sakai is known only from Japan.

Our grateful thanks are due to Captain K. L. Chopra, Commanding Officer, I.N.S. Darshak, for help in the collection of samples and to Dr. S. Khera, Joint Director-in-Charge, Zoological Survey of India, Calcutta, for facilities given to participate in the Expedition.

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February 21, 1977.

A. DANIEL.
S. CHAKRAPANY.

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1. Alcock, A., *J. Asiat. Soc. Beng.*, 1896, **65**, Part II (2), 134.
2. Laurie, R. D., *J. Linn. Soc.* (*Zool.*), 1915, **31**, 407.
3. Tyndale-Biscoe, M. and George, R. W., *J. Roy. Soc. West. Aust.*, 1962, 45, Part 3, 65.
4. Ihle, J. E. W., *Siboga Exped. Monogr.*, 39b², 1918, p. 1.
5. Sakai, T., *Sci. Rep. Tokyo Bunrika Diag. Sectn. B*, 3 Supp., 1937, **2**, 67.
6. Yokoya, Yu, *J. Coll. Agric.*, Tokyo, 1933, **12**, 1.
7. Sakai, T., *Rec. Oceanogr. Wks. Jap.*, 1940, **2** (1), 27.
8. Holthuis, L. B., In *Rumphius Memorial Volume*, 1959, p. 63 (Leiden).
9. —, *Bull. Zool. Nom.*, 1962, **19** (1), 51.
10. Utinomi, H., *Coloured Illustrations of Sea Shore Animals of Japan* (Hoikusha: Osaka), 1960.

6435/77. Published by S. R. S. Sastry, Manager, Current Science Association, Bangalore-560006
and Printed at The Bangalore Press, Bangalore-560018.

A NEW APPROACH TO REPULSION IN IONIC CRYSTALS

II. Further Refinement on the Alkali Halides

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ABSTRACT

The repulsion parameters in the formulation by the authors (*J. Phys. Chem. Solids*, 1976, **37**, 395; *Curr. Sci.*, 1976, **45**, 357) for the alkali and the halogen ions have been refined using more experimental data. These parameters could be used to calculate the repulsion potential in any crystal of any structure in which these ions may occur. The r.m.s. error in the alkali halides between the measured and calculated values of the nearest neighbour distance is 0·712% and that in $d^2 W_L/dr^2$ is 7·40%. The corresponding values in the theory of Tosi and Fumi (*J. Phys. Chem. Solids*, 1964, **25**, 45) are 1·381% and 6·06%. Our earlier claim that our values were much better than Tosi and Fumi arose out of a computational error and is hence retracted here. Our present repulsion parameters indicate that the alkali ions are (as is to be expected) much less compressible compared to the halogen ions.

RECENTLY we proposed a new approach to repulsion in ionic crystals[1-3] based on the following three postulates:

(*a*) A specific compression energy is associated with each ion and the repulsion between ions arises *solely* from the resistance of the ions to compression.

(*b*) The compression energy function of an ion is independent of the neighbouring ions that compress it.

(*c*) The compression energy of an ion is directly proportional to the number of nearest neighbours.

In the present formulation of repulsion, the compression energy function of an ion that has been determined in one crystal in one structure, can be used for that ion in any other crystal (of any other structure) in which it occurs. This lends predictive power to our approach, in contrast to earlier approaches to repulsion which require the refinement of new parameters in each new crystal.

For the compression energy of the ion, the specific exponential form ($Ae^{-r/\rho}$) was chosen arbitrarily[2], where r is the radius of the ion and A and ρ are repulsion parameters to be refined for each ion. In this form, 18 parameters are enough to describe all the 20 alkali halides in all their structural modifications and over a range of pressures. In contrast, the widely accepted repulsion potentials of Tosi and Fumi[4] require 27 parameters to describe just the atmospheric pressure behaviour of 17 alkali halides (they omit CsCl, CsBr and CsI).

The refinement of the repulsion parameters A and ρ for the alkali and the halogen ions using the data on the alkali halides involves the simultaneous optimization of a large number of strongly correlated parameters. The parameters presented earlier[2,3] were obtained using a graphical method. Although the refinement there was not complete, those parameters are sufficiently accurate for the calculation of repulsion potentials. Further refinement was, however, undertaken because of our interest in the correlations among the repulsion parameters of different ions. In addition to the experimental data used earlier[2], new high pressure data[5,6] on LiF and NaF were also included. We shall not discuss here the actual procedure followed for the refinement except to mention that the computations were quite tedious and the convergence very slow.

Table 1 lists the repulsion parameters obtained for the alkali and the halogen ions. The values of A and ρ given in papers (2) and (3) are now to be replaced by the values given here. The r.m.s. error, for all the alkali halides, between the measured values of r, the nearest neighbour distance, and the values calculated with the repulsion parameters of Table 1, is 0·712%. The r.m.s. error in d^2W_L/dr^2, which depends on the bulk modulus, is 7·40%. The corresponding r.m.s. errors in the values predicted by the potentials of

TABLE 1

Repulsion parameters for the alkali and the halogen ions

Ion	A (erg)	ρ (Å)
Li^+	$0 \cdot 1544 \times 10^{-5}$	0·04158
Na^+	$0 \cdot 1211 \times 10^{-7}$	0·09029
K^+	$0 \cdot 5600 \times 10^{-7}$	0·1078
Rb^+	$0 \cdot 3980 \times 10^{-6}$	0·08883
Cs^+	$0 \cdot 5604 \times 10^{-6}$	0·09977
F^-	$0 \cdot 7506 \times 10^{-10}$	0·2152
Cl^-	$0 \cdot 2958 \times 10^{-9}$	0·2240
Br^-	$0 \cdot 3724 \times 10^{-9}$	0·2352
I^-	$0 \cdot 4407 \times 10^{-9}$	0·2538

TABLE II

Ionic radii (in Ångstroms) in the alkali halides at atmospheric pressure

	F^-		Cl^-		Br^-		I^-	
Li^+	0·727	1·271	0·760	1·800	0·770	1·990	0·786	2·270
Na^+	1·066	1·259	1·105	1·706	1·111	1·849	1·144	2·114
K^+	1·425	1·272	1·457	1·688	1·461	1·826	1·481	2·040
Rb^+	1·563	1·255	1·598	1·693	1·610	1·851	1·611	2·025
Cs^+	1·766	1·230	1·844	1·752	1·845	1·886	1·846	2·060

Tosi and Fumi[4] are 1·381% for r and 6·06% for d^2W_L/dr^2.

In earlier papers[2,3] we claimed that the r.m.s. fit in r and d^2W_L/dr^2 obtained with our potentials is superior to that obtained with the potentials of Tosi and Fumi[4]. This claim is not correct since the r.m.s. errors (for the potentials of Tosi and Fumi) 1·435% and 15·40% quoted there arose from a computational error. The present comparison indicates that the fit in the two theories are of the same order. However, the advantages of the present formulation mentioned at the beginning of this paper are still valid.

The repulsion parameters listed in Table I are different from those listed in references 2 and 3. However, the ionic radii (listed in Table II) predicted by the new set of parameters are not significantly different. During the refinement, the radii of the ions remained almost the same while the compressibility of the ions, which is qualitatively measured by the parameter ρ, varied considerably. The essential difference of the present set of repulsion parameters from the earlier set[2,3] is that the alkali ions are much harder and the halogen ions much softer than predicted by the earlier parameters.

As a consequence, the present repulsion parameters indicate that the alkali ions are much less compressible than the halogen ions. For instance, calculations show that in CsI, between 0 and 40 kbars, the radius of Cs^+ decreases by 0·062 Å while the radius of I^- decreases by 0·159 Å. In LiF, between 0 and 150 kbars, the radius of Li^+ decreases by only 0·017 Å while the radius of F^- decreases by only 0·017 Å while the radius of F^- consistent with the relatively large polarizabilities of the halogen ions. The compressibilities of ions will be discussed in some detail in a later communication.

ACKNOWLEDGEMENT

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1. Narayan, R. and Ramaseshan, S., *Pramāṇa*, 1974, 3, 143.
2. — and —, *J. Phys. Chem. Solids*, 1976, 37, 395.
3. Ramaseshan, S. and Narayan, R., *Curr. Sci.*, 1976, 45, 357.
4. Tosi, M. P. and Fumi, F. G., *J. Phys. Chem. Solids*, 1964, 25, 45.
5. Pagannone, M. and Drickamer, H. G., *J. Chem. Phys.*, 1965, 43, 2266.
6. Vaidya, S. N. and Kennedy, G. C., *J. Phys. Chem. Solids*, 1971, 32, 951.

JAWAHARLAL NEHRU FELLOWSHIP

Dr. S. Ramaseshan, Head of the Materials Division, National Aeronautical Laboratory, Bangalore, has been awarded the Jawaharlal Nehru Fellowship. During the tenure of the Fellowship, Dr. Ramaseshan will work on 'The Life and Work of Prof. Sir C. V. Raman' covering the biographical aspects, his scientific work and its impact on Indian Science.

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SEMIEMPIRICAL INVESTIGATIONS OF LINDERBERG'S RELATION

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ABSTRACT

Semiempirical calculation on singlet transition energies of several aromatic hydrocarbons is carried out using a modified form of Linderberg's relation for the resonance integral in the Pariser, Parr, Pople theory. The results are interpreted in terms of the behaviour exhibited by some model one-electron system. It is concluded that the original Linderberg relation is essentially valid for this group of molecules.

INTRODUCTION

THE relation

$$\beta = \frac{1}{R}\frac{dS}{dR} \qquad (1)$$

here β is the resonance integral, S the overlap inte- ral and R the distance separating the orbital centre as proposed by Linderberg[1] by invoking a consis- ncy requirement in the Pariser, Parr, Pople (PPP) eory. Examination of two-centre one-electron model stems has revealed[2,3] that the validity of the lation crucially depends on the form of the e-centre potential. For example, the relation inapplicable if the potential is the infinite range ulomb interaction. However, if the potential is a perposition of screened and unscreened coulomb teractions, then although the relation is not valid general, there exists a region characterised by the reening parameter and the distance R where the tio

$$\beta \Big/ \frac{1}{R}\frac{dS}{dR} \quad \text{(equal to say, K)}$$

slowly varying and lies within 20% of unity. The odel systems imply that different situations— mely (i) the relation is inapplicable, (ii) e relation is applicable with (*a*) K different m unity and (*b*) K equal to unity—can be expected actual molecular systems. The decisions between se possibilities being difficult *a-priori*, an extensive ies of calculations have been carried out to examine e matter *a-posteori*. We report here the results tained on hydrocarbons; the results on molecules ntaining heteroatoms shall be dealt with later.

TABLE I.

Singlet transition energies (ev) of hydrocarbons using Slater orbitals (oscillator strengths are given in parenthesis)

Molecule	Calculated	Experimental
Benzene	4·63 (0·00)	4·72 (0·00)[8]
	5·93 (0·00)	5·90 (0·10)
	6·75 (1·15)	6·76 (0·69)
	6·75 (1·15)	
Naphthalene	3·93 (0·00)	3·97 (0·002)[9]
	4·33 (0·23)	4·29 (0·18)
	5·61 (2·00)	5·64 (1·70)
	6·18 (0·57)	6·53 (0·20)
Anthracene	3·42 (0·35)	3·28 (0·10)[9]
	4·92 (2·65)	4·88 (2·28)
	5·61 (0·01)	5·61 (0·28)
	6·10 (0·30)	
Naphthacene	2·85 (0·39)	2·62 (0·08)[9]
	3·48 (0·02)	
	4·50 (3·19)	4·56 (1·85)
	5·64 (0·10)	5·88 (0·45)
Pentacene	2·49 (0·42)	2·43[9]
	3·40 (0·70)	
	4·23 (3·59)	4·00
	4·24 (0·10)	
Phenanthrene	3·74 (0·00)	3·76 (0·003)[9]
	4·16 (0·41)	4·23 (0·18)
	5·02 (1·11)	4·93 (1·09)
	5·71 (0·43)	5·84 (0·60)

METHOD

Conventional PPP formalism was employed for culating the singlet transition energies of the eleven drocarbons listed in Tables I and II. The various egrals, with the exception of the resonance integral,

TABLE I—*Contd.*

Molecules	Calculated	Experimental
Chrysene	3·63 (0·03)	3·41 (0·005)[9]
	3·76 (0·64)	3·74 (0·36)
	4·79 (2·12)	4·61 (1·29)
	5·18 (0·86)	5·64 (0·69)
Pyrene	3·52 (0·81)	3·34[10]
	3·55 (0·00)	3·70
	4·35 (0·00)	
	4·42 (0·00)	4·45
	4·83 (1·18)	
	5·14 (0·00)	5·15
Perylene	2·95 (1·03)	2·86 (0·33)[11,12]
	5·04 (1·82)	4·91 (0·44)
		5·07 (0·44)
	6·01 (1·12)	6·01 (2·17)
	6·56 (1·97)	
Coronene	3·14 (0·00)	3·14[13]
	3·51 (0·00)	3·69
	4·29 (2·26)	
	4·29 (2·26)	4·07
Azulene	1·82 (0·02)	1·79 (0·009)[14]
	3·34 (0·01)	3·50 (0·08)
	4·22 (0·09)	4·05 (?)
	4·59 (1·75)	4·52 (1·10)
	5·50 (0·41)	5·24 (0·38)

were evaluated using the well-known and generally accepted formulas[4]. For example, the coulomb integral was estimated *via* the Goeppert Mayer-Sklar approximation with neglect of penetration integrals. The two-centre repulsion integrals were calculated from the Nishimoto-Mataga formula. Limited configuration interaction was invoked involving single excitations from the four highest occupied orbitals to the four lowest occupied orbitals.

The resonance integral was computed from a modified form of (1) namely

$$\beta = \frac{K}{R}\frac{dS}{dR} \qquad (2)$$

where K is an adjustable empirical parameter. The overlap integral S was evaluated using Slater orbitals and Mulliken's formula[5].

RESULTS AND DISCUSSION

Actual bond distances were employed in the first set of calculations. Singlet transition energies were computed using a range of K values and root mean square deviation (σ) was obtained for each value of K.

TABLE II

Singlet transition energies (ev) of hydrocarbons using symmetrically orthogonal orbitals (oscillator strengths are given in parenthesis).

Molecule	Calculated
Benzene	4·90 (0·00), 6·20 (0·00), 7·03 (1·19), 7·03 (1·19)
Naphthalene	4·16 (0·00), 4·50 (0·25), 5·83 (2·09), 6·45 (0·60)
Anthracene	3·54 (0·36), 5·12 (2·85), 6·33 (0·31), 7·09 (1·19)
Naphthacene	2·92 (0·38), 3·94 (0·00), 4·71 (3·20), 5·02 (0·07)
Pentacene	2·54 (0·41), 3·48 (0·00), 4·37 (0·14), 4·44 (3·57)
Phenanthrene	3·89 (0·00), 4·33 (0·46), 5·21 (0·63), 5·91 (0·42)
Chrysene	3·73 (0·00), 3·91 (0·69), 4·95 (2·34), 5·37 (0·91)
Pyrene	3·65 (0·88), 3·73 (0·00), 4·55 (0·00), 4·64 (0·00), 4·98 (1·08), 5·21 (0·00)
Perylene	3·04 (1·09), 5·23 (1·91), 6·28 (1·16), 6·83 (2·03)
Coronene	3·29 (0·00), 3·66 (0·00), 4·44 (2·36), 4·44 (2·36)
Azulene	1·93 (0·02), 3·50 (0·01), 4·44 (0·09), 4·76 (1·82), 5·75(0·46)

The plot of σ *versus* K (Fig. 1) shows a minimum at K = 0·95. Since it is often convenient to

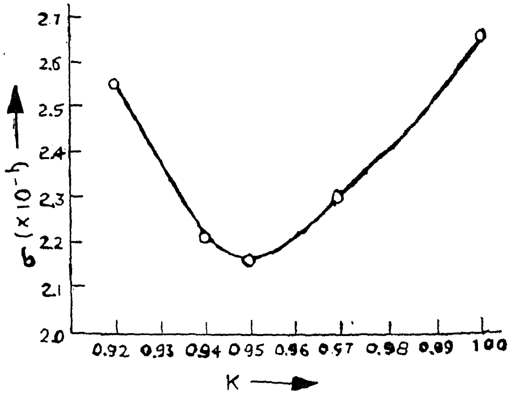

FIG. 1, Variation of σ with K.

use idealised geometry rather than the actual one, calculations were repeated in the second set with all the C-C bonds taken as 1·40 Å. In this case the value of K at the minimum was found to be 0·99. The fact that K is not significantly different from unity means that the original Linderberg relation [equation (1)] should be a useful formula for evaluating the resonance integral for hydrocarbons. The calculated values of the singlet transition energies with $K = 1$ are given in Table I.

The derivation of (1) is based on the use of an orthonormal basis set. To check the effect of changing the basis from non-orthogonal to orthogonal one, calculations were next performed employing symmetrically orthogonal orbitals. The results given in Table II for $K = 1$ show that the agreement is not unsatisfactory. It thus appears that the applicability of equation (1) is not seriously influenced by minor variations in geometry and the nature of orbitals.

It must be pointed out that the modified Linderberg relation of form (2) was first proposed by De Bruijn[6]. He argued that since the PPP hamiltonian does not include the polarisation of the σ-framework and the π-electron wavefunction does not include the full correlation effects, the β value empirically chosen to compensate for these errors need not necessarily agree with those calculated from (1). By proceeding to include the effects due to σ electrons, he arrived at equation (2) where K depends on the properties of the σ core, the extent of configuration interaction, as well as on the type of atomic orbitals used. Our choice of (2), however, is based on totally different considerations. As stated in the Introduction, Linderberg's relation has been shown to be inapplicable in even one-electron two-centre model systems (where correlation effects are, of course, missing) if the one-centre potential is of infinite range type. Only if the potential has a strong short range component, an equation of the form (2) becomes applicable. In as much as the effective potential on a π-electron should be of short range type due to screening effects, the applicability of (2) to conjugated molecules is quite understandable. However there are no *a-priari* grounds for K to be equal to 1. The fact that $K \approx 1$ nicely fits the hydrocarbon data means that the effective potential has such a character that the original Linderberg relation happens to be adequate in this particular case.

The validity of (1) in the PPP model was first investigated by Bailey[7] by direct calculation on large number of molecules. However, the hydrocarbons (with the exception of benzene) had not been considered in that work. Our results show that the original Linderberg relation applies particularly well to this group of conjugated molecules.

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1. Linderberg, J., *Chem. Physics Letters*, 1967, **1**, 39.
2. Kapur (nee Sane), K., Sane, K. V. and Srivastava, P. K., *Int. J. Quant. Chem.*, 1973, 7, 1029.
3. Raina, V., Srivastava, P. K., and Sane, K. V., *Chem. Physics Letters*, 1976, **42**, 517.
4. (*a*) Parr, R. G., *Quantum Theory of Molecular Electronic Structure*, Benjamin, W. A., Inc., 1964.
   (*b*) Flurry, R. L., *Molecular Orbital Theories of Bonding in Organic Molecules*, Marcel Dekker, Inc., 1968.
5. Mulliken, R. S., *Phys. Rev.*, 1928, **32**, 186; 1928, **32**, 761; 1929, **33**, 730.
6. De Bruijn, S., *Chem. Physics Letters*, 1971, **8**, 19.
7. Bailey, M. L., *Theoret. Chim. Acta* (*Berl.*,) 1969, **13**, 56; 1970, **16**, 306.
8. Petruska, J., *J. Chem. Phys.*, 1961, **34**, 1120.
9. Klevens, H. B. and Platt, J. R., *Ibid.*, 1949, **17**, 470.
10. Jaffe, H. H. and Orchin, M. *Theory and Applications of U.V. Spectroscopy*. J. Wiley and Sons, Inc., New York; 1962.
11. Clar, E., *Spectrochim. Acta*, 1950, **4**, 119.
12. Tanaka, J., *Bull. Chem. Soc. Japan*, 1963, 36, 1237.
13. Experimental data based on spectra in Friedel, R. A. and Orchin, M., *U.V. Spectra of Aromatic Compounds*, New York, J. Wiley and Sons, Inc., 1951.
14. Mann, Platt and Klevens: *J. Chem. Phys.*, 1949, 1[illegible], 481.

INDIAN NATIONAL SCIENCE ACADEMY, NEW DELHI-1

The Indian National Science Academy in association with the International Union of Theoretical and Applied Mechanics (IUTAM) and the International Union of Geodesy and Geophysics (IUGG) is organising an International Symposium on 'Monsoon Dynamics' with application to weather forecasting and flood prediction, at the Indian Institute of Technology, Delhi, from 5–9 December 1977.

For further details please contact Shri S. K. Sahni, Project Coordinator, Indian National Science Academy, New Delhi-1.

STUDIES IN *CATHARANTHUS ROSEUS* CALLUS CULTURES, CALLUS INITIATION AND DIFFERENTIATION

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THE plants of *Catharanthus* species have been reported to have about eighty alkaloids of which vincrystine and vinblastine are being utilized as antitumor drugs. The callus cultures of *C. roseus* (L) G. Don produce ajamalacine, vindoline, vindoniline and other alkaloids classified into seven structural types[1]. Recently Scott and Lee[2] have demonstrated the presence of enzymes in *C. roseus* callus that catalyse the synthesis of corynanthe alkaloids. The presence of vincrystine and vinblastine in callus cultures has not, however, been reported. The observations of West and Mika with *Atropa belladone* callus[3], Newmann with *Macleya cordata* callus[4], Newmann and Muller[5] and Tabata and Hiraoka[6] with *Nicotiana* callus have indicated that differentiation of callus, specially into root, enhances alkaloid synthesis. It has not, however, been shown whether synthesis of vinblastine and vincrystine could be stimulated in callus under conditions favourable for differentiation. This report deals with standardization of the conditions for *C. roseus* viable callus cultures and the differentiation *in vitro*.

The leaf and shoot callus cultures of two varieties of *C. roseus*, one with pink flower (VR) and another with white flower (VA) were initiated *in vitro* following the method of White[7]. The leaf callus in both the varieties (VAl and VRl) were cultivated on half strength Murashige and Skoog's[8] mineral solution, vitamins, 2, 4-dichlorophenoxy acetic acid (2, 4–D) and coconut milk (CM). The shoot callus in both the varieties (VAs and VRs) were initiated on Murashige and Skoog's basal medium (MSB) containing naphthalene-acetic acid (NAA) and kinetin with or without casein hydrolysate.

The VAl callus was best maintained in MSB with kinetin (1mg/l) and 2·4–D (0·5 mg/l). The callus could grow without auxin or cytokinin, but, addition of these two separately or together increased the growth by 2 to 10 fold. NAA gave equally good growth when percentage increase in dry weight of callus was compared. 6-Benzylaminopurine, kinetin and zeatin, independently, enhanced the growth (Table I) when dry weights were compared. The correlation between light conditions and auxin concentration was quite striking in this callus. When callus was grown in dark and sub-cultured into media having low concentrations (0·2 mg/l) of auxine it did not survive, but at high concentrations of auxins (·5 and 1 mg/l) grew profusely. The light grown callus, on the other hand, had no such remarkable change in growth pattern at low or high levels of auxins (Figs. 1 and 2, Tubes 2 and 4). Root-like projections or callus grown on NAA containing media were observed.

TABLE I

Effect of different auxins and cytokinins on the growth of C. roseus *callus cultures*

Medium: Basal medium of Murashige and Skoog's auxins and cytokinins added as mentioned in the table.
Inoculum: 80 mg fresh wt/Tube.

Sl. No.	Medium	*% increase over the inoculum in mg	% dry wt in mg
1.	Basal medium (BM)	125	11·1
2.	BM + 2, 4-D (0·5 mg/l)	226	8·2
3.	BM + Kinetin (1 mg/l)	422	11·5
4.	BM + 2, 4-D (0·5 mg/l) + Kinetin (1 mg/l)	1386	5·4
5.	BM + 2, 4-D (0·5 mg/l) + BAP (1 mg/l)	1816	4·7
6.	BM + 2, 4-D (0·5 mg/l) + Zeatin (10 mg/l)	731	7·0
7.	BM + NAA (0·5 mg/l) + Kintetin (1 mg/l)	766	9·0
8.	BM + IAA (0·5 mg/l) + Kinetin (1 mg/l)	410	10·25

$$* \ \frac{\text{Total wt.} - \text{Inoculum wt.}}{\text{Inoculum wt.}} \times 100.$$

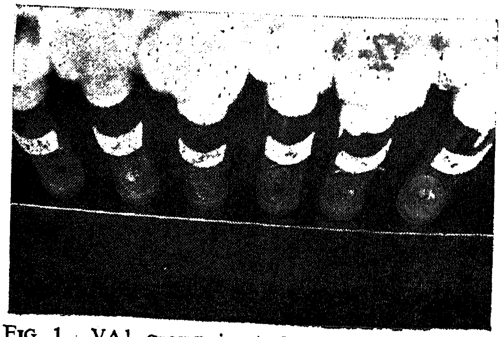

FIG. 1. VA1 grown in dark and subcultured into different concentrations of auxins. Growth after 25 days. Tubes 1, 3 and 5 contain NAA and Tubes 2, 4 and 6 contain 2, 4-D at 0·2, 0·5 and 1·0 mg/l concentrations respectively.

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The VR1 callus was observed to grow well on Schenk and Hildebrandt's basal medium (SHB)[9], containing indole acetic acid (IAA) and kinetin (1 mg/l each). This callus was brown and light, had no specific effect on its growth. The callus could differentiate into roots at high concentration of NAA (2·5 mg/l).

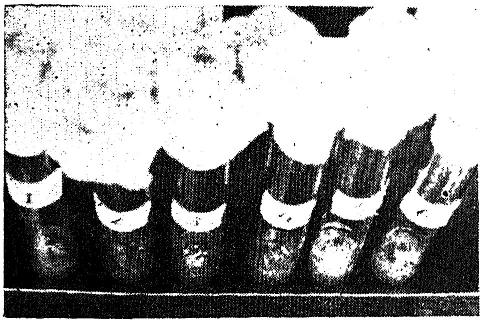

FIG. 2. VA1 grown in light and subcultured into different concentrations of auxins (details as in Fig. 1).

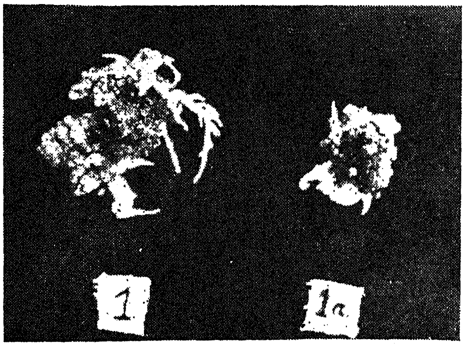

FIG. 3. Growth and rooting in VRs. 1. NAA-1 mg/l; 1 *a*. NAA–0·1 mg/l.

The SHB media containing kinetin and 2, 4-D or NAA supported viable callus cultures of both the varieties. The VAs cultures were white, soft and were grown on 2, 4-D containing media. When subcultured on NAA (2·5 mg/l) containing media 25% of the inoculum on this media differentiated into root-like structures.

The VRs callus differentiated very well into roots on NAA containing media. This differentiating capacity was not lost on repeated subcultures. In spite of the poor growth at low levels of NAA (0·1 mg/l), it still retained the capacity for rooting (Fig. 3). Of the auxins studied, rooting was observed in IAA containing media but was not as much as in the presence of NAA. 2, 4-D inhibited differentiation at the concentrations studied (1 mg/l).

It is hoped that the standardization of optimum conditions for the growth and differentiation of callus cultures of *C. roseus*, reported here, would pave the way for further investigations on the biosynthesis of antitumor alkaloids.

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1. Krikorian, A. D. and Steward, F. C., Cited in *Plant Physiology*, Ed. F. C. Steward, Academic Press, New York, 1969, **5B**, 227.
2. Scott, A. I. and Lee, Siu Leong, *J. Am. Chem. Soc.*, 1975, **97**, 6906.
3. West, F. and Mika, E., *Bot. Gaz.*, 1959, **119**, 50.
4. Newmann, D., *Kut'l Izalirovonnykh Organor Thanei Kletoh Rast Tr. Vses. Konf.* 1*st*, Ed. Butenko R. G., 1970.
5. Newmann, D. and Miller, E., *Biochem. Physiol. Pflanz.*, 1971, **162** (6), 503.
6. Tabata, M. and Hiraoka, N., *Physiol Plant.*, 1976, **38**, 19.
7. White, P. R., Cited in *The Cultivation of Animal and Plant Cells*, Ronald Press. N.Y.
8. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 474.
9. Schenk, R. Y. and Hildebrandt, A. C., *Can. J. Bot.*, 1972, **50**, 199.

INDIAN CHEMICAL SOCIETY—SEMINAR ON ORGANOMETALLIC CHEMISTRY

A three-day Seminar on 'Organometallic Chemistry' sponsored by the University Grants Commission will be held in the Chemistry Department of Lucknow University from Monday, October 10 to Wednesday, October 12, 1977. The programme will include six invited Session Lectures and approximately twenty-five contributed papers covering synthesis, structure, bonding, reactions and biological or chemical uses of metal-carbon bonded compounds.

Further enquiries may be had from the Convener, Seminar on Organometallic Chemistry, Department of Chemistry, University of Lucknow, Lucknow-226007, latest by Wednesday, 15th June, 1977.

ANTICONVULSANT AND SUCCINATE DEHYDROGENASE INHIBITORY ACTIVITY OF SOME NEWER THIOBARBITURATES

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Jawaharlal Nehru Laboratory of Molecular Biology, Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow, India

ABSTRACT

Some 1-aryl-3-cyclohexyl thiobarbiturates were synthesized and investigated for their ability to inhibit succinate dehydrogenase (SDH) activity of rat brain homogenate. All compounds exhibited concentration dependent inhibition of enzyme. The degree of enzyme inhibition was also evaluated on the basis of their I_{50} and $I_{50}/(S)$ values. Kinetic study carried out with 1-(2-ethoxyphenyl)-3-cyclohexyl-thiobarbiturate revealed a competitive nature of inhibition. Anticonvulsant activity of the compounds against pentylenetetrazol-induced seizures and the partition coefficients were determined in order to correlate structure-activity relationship.

INTRODUCTION

NUMEROUS CNS depressants and anticonvulsants have been shown to inhibit respiratory activity of brain which ultimately leads to altered energy metabolism and thus impair neuronal functions.[1–3] Furthermore, it has been reported that various barbiturates interfere with the succinate dehydrogenase activity[4–6]. Recently, several 1, 3-disubstituted thiobarbiturates were investigated to show their effects on respiratory enzymes, convulsive seizures and succinate dehydrogenase activity of rat brain homogenate[7,8]. On the basis of these observations, a few 1-aryl-3-cyclohexyl-thiobarbiturates were synthesized and investigated for succinate dehydrogenase activity *in vitro* of rat brain homogenate. The compounds were screened for anticonvulsant activity and the partition coefficients were also determined in order to discover any relationship between structure, biological activity and lipophilicity.

EXPERIMENTAL

1-Aryl-3-cyclohexyl thiocarbamides.—Cyclohexylamine (0·01 mole) and the appropriate aryl isothiocyanate (0·01 mole) were taken in 20 ml of dry benzene and refluxed on a steam bath for 2 hr. The reaction mixture was then concentrated under reduced pressure. The solid mass which separated out on cooling was filtered, washed (Et_2O, dil. HCl), dried and recrystallized from EtOH. All thiocarbamides have been reported earlier[9] except 1-(2-ethoxyphenyl)-3-cyclohexyl thiocarbamide m.p. 104° C, yield 82%. Analyses for $C_{15}H_{22}N_2OS$

Calcd. C, 64·74%; H, 8·27%; N, 10·07%.

Found C, 64·46%; H, 8·4%; N, 10·32% and

1-(4-ethoxyphenyl)-3-cyclohexyl thiocarbamide, m.p. 125° C, yield 80%. Analyses for $C_{15}H_{22}N_2OS$

Calcd. C, 64·74%; H, 8·27%; N, 10·07%

Found C, 64·86%; H, 8·15%; N, 9·80%.

1-Aryl-3-cyclohexyl thiobarbiturates

Appropriate thiocarbamide (0·001 mole) and malonic acid (0·015 mole) were heated slowly on a water bath with acetyl chloride (7 ml) at 60–80° for 1 hr. After cooling, the pasty mass was triturated with water and the brown solid was separated and recrystallized from ethanol. All thiobarbiturates were characterized by their sharp melting points, elemental analyses (Table I) and infrared spectra.

Succinate dehydrogenase activity.—Succinate dehydrogenase activity of rat brain homogenate was determined by the spectrophotometric method described by Slater and Bonner[10].

Determination of I_{50} value.—The concentration of the compound required to inhibit 50% of the enzyme activity was obtained by plotting a graph of log molar concentration of compounds against per cent inhibition.

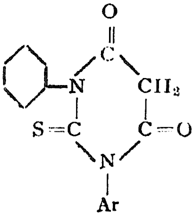
Anticonvulsant activity.—Anticonvulsant activity of 1-aryl-3-cyclohexyl thiobarbiturates was determined at a dose of 100 mg/kg i.p. against pentylenetetrazol-induced seizures[11].

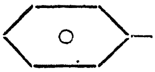
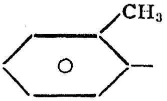
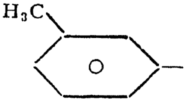
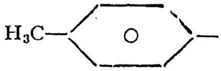
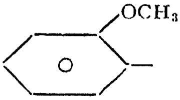
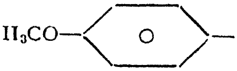
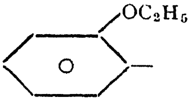
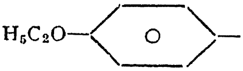
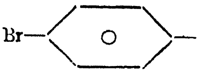
Determination of partition coefficients.—The partition coefficients were determined by dissolving the compounds in chloroform, shaking the solution with water and assaying the concentration of the compound in two phases spectrophotometrically.

RESULTS AND DISCUSSION

All 1-aryl-3-cyclohexyl thiobarbiturates were found to inhibit rat brain succinate dehydrogenase *in vitro* at final concentrations of $1 \times 10^{-3} M$, $5 \cdot 0 \times 10^{-4} M$, $2 \cdot 5 \times 10^{-4} M$, $1 \times 10^{-4} M$ and $5 \times 10^{-5} M$ (Table II). The degree of inhibition of succinate dehydrogenase was found to be concentration dependent. This necessitated the determination of I_{50} values of the thiobarbiturates and the ratio of I_{50} values to substrate concentration (Table III) for a better comparative evaluation of their inhibitory activity. 1-(2-Ethoxyphenyl)-3-cyclohexyl thiobarbiturate (Compound VII) exhibited maximum inhibition.

TABLE I
Physical constants of 1-*oryl*-3-*cyclonexyl thiobarbiturates*

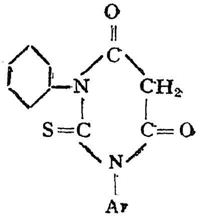


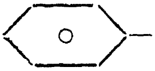
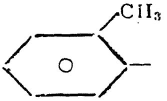
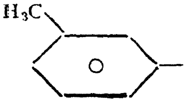
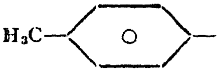
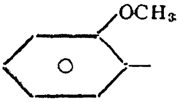
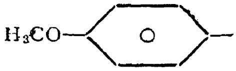
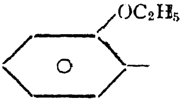
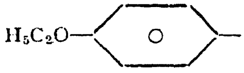
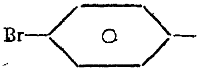
Compound No.	Ar.	Melting point	Yield %	Molecular formula	Analysis %		
						Calcd.	Found
I		124°G	72	$C_{16}H_{18}N_2O_2S$	C H N	63·57 5·96 9·27	63·78 6·01 9·15
II		132° C	68	$C_{17}H_{20}N_2O_2S$	C H N	64·56 6·33 8·86	63·36 6·29 9·06
III		182° C	70	$C_{17}H_{20}N_2O_2S$	C H N	64·56 6·33 8·86	64·85 6·47 8·65
IV		90° C	76	$C_{17}H_{20}N_2O_2S$	C H N	64·56 6·33 8·86	64·68 6·42 8·99
V		142–144° C	64	$C_{17}H_{20}N_2O_3S$	C H N	61·44 6·02 8·43	61·73 5·96 8·68
VI		152° C	68	$C_{17}H_{20}N_2O_3S$	C H N	61·44 6·02 8·43	61·27 6·23 8·36
VII		138° C	71	$C_{18}H_{22}N_2O_3S$	C H N	62·43 6·36 8·09	62·57 6·42 8·27
VIII		158° C	63	$C_{18}H_{22}N_2O_3S$	C H N	62·43 6·46 8·09	62·20 6·48 8·15
IX		148° C	64	$C_{16}H_{17}N_2O_2SBr$	C H N	53·54 4·46 7·34	53·79 4·28 7·55

Substitution of various groups at phenyl ring attached to position 1 of the thiobarbiturate nucleus was found to elicit a varied degree of inhibition of SDH activity. The shifting of methoxy/ethoxy group from position 2 to 4 (Compounds, V, VI, VII, VIII) resulted in the decrease in the enzyme inhibition. Moreover, the compounds possessing ethoxy groups were found to show greater activity as compared to those having methoxy groups at the respective positions in the phenyl ring. The introduction of an

TABLE II

Effect of 1-aryl-3-cyclohexyl-thiobarbiturates on rat brain succinate dehydrogenase



Compound No.	Ar	$1 \times 10^{-3} M^a$	Per cent inhibition*			
			$5 \cdot 0 \times 10^{-4} M^b$	$2 \cdot 5 \times 10^{-4} M^c$	$1 \times 10^{-4} M^d$	$5 \times 10^{-5} M^e$
I		58·0±0·3	33·0±0·8	26·9±0·7	20·8±0·9	14·4±0·6
II		70·0±0·08	41·4±0·9	32·1±1·0	22·3±0·4	5·8±0·7
III		72·0±0·1	62·9±0·7	33·8±0·5	31·3±0·6	12·7±0·9
IV		76·0±1·0	51·4±0·6	35·7±0·7	28·3±0·8	2·90±0·4
V		92·3±1·0	61·4±0·2	42·8±0·8	38·8±0·6	17·4±0·8
VI		70·7±0·2	51·4±0·8	33·8±0·4	23·8±0·9	12·7±1·0
VII		98·2±0·4	66·1±0·8	58·5±0·8	47·7±0·7	21·2±0·6
VIII		94·8±0·3	51·4±0·5	54·6±0·3	29·8±0·5	12·6±0·8
IX		92·1±0·5	47·1±0·3	44·6±0·8	25·3±1·3	12·6±0·9

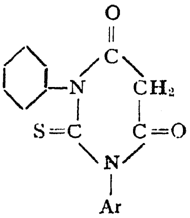
* Assay procedures and contents of the reaction mixture were as described in the text. Each experiment was done in triplicate and the values are the mean values of three separate experiments with ± standard error of the mean.

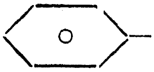
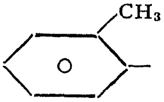
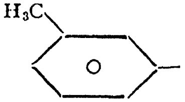
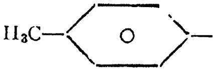
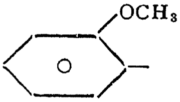
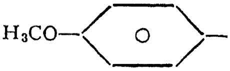
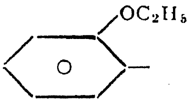
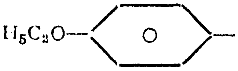
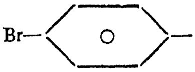
a, b, c, d, e — Indicate the final concentration of the test compound used in the reaction mixture.

electronegative Br atom in the phenyl ring (Compound IX) exhibited a similar degree of inhibition as compared to the electron-donating methoxy and ethoxy groups. In the case of methyl substituted barbiturates (II, III and IV), the degree of inhibition was found to be in order of $-p > -m > -o$ at the final concentrations of $1 \times 10^{-3} M$ and $2 \cdot 5 \times 10^{-4} M$. The order $-m > -p > -o$ was observed at the final concentration of $5 \times 10^{-4} M$ and $1 \times 10^{-4} M$ while the order was $-m > -o > p$ at $5 \times 10^{-5} M$. However, the compound possessing an unsubstituted phenyl ring exhibited minimum inhibition at various con-

TABLE III

I_{50} values of 1-aryl-3-cyclohexyl thiobarbiturates



Compound No.	Ar.	I_{50}*	I_{50}** (S)
I		$8\cdot4 \times 10^{-4}$ *M*	0·168
II		$6\cdot6 \times 10^{-4}$ *M*	0·132
III		$3\cdot8 \times 10^{-4}$ *M*	0·076
IV		$4\cdot8 \times 10^{-4}$ *M*	0·096
V		$3\cdot4 \times 10^{-4}$ *M*	0·068
VI		$4\cdot9 \times 10^{-4}$ *M*	0·098
VII		$1\cdot2 \times 10^{-4}$ *M*	0·024
VIII		$4\cdot5 \times 10^{-4}$ *M*	0·090
IX		$5\cdot4 \times 10^{-4}$ *M*	0·108

* Represents the molar concentration of inhibitor causing 50% inhibition of enzyme under the experimental conditions described in the text.

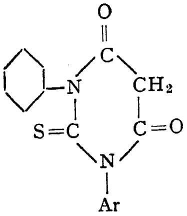
** Indicates the ratio of molar concentration of compounds to substrate giving 50% inhibition.

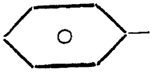
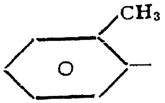
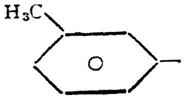
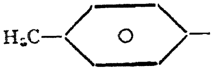
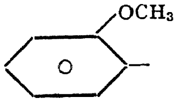
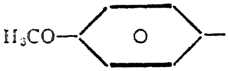
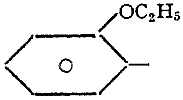
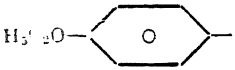
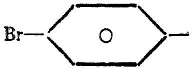
concentrations. Preincubation of these compounds with the enzyme system for varying lengths of time prior to the addition of substrate in no way altered their degree of inhibition (Table IV). Kinetic study of 1-(2-ethoxyphenyl)-3-cyclohexyl thiobarbiturate using Lineweaver and Burk[12] plots as modified by Dixon[13] has been demonstrated in Fig. 1. Ki value obtained graphically, was found to be $0\cdot125 \times 10^{-4}$ *M*.

All thiobarbiturates afforded protection (10%–60%) aganist pentylenetetrazol-induced seizures (Table V). Maximum protection was shown by compound IV while the minimum was exhibited by compound I. In general,

TABLE IV

Preincubation studies with 1-aryl-3-cyclohexyl thiobarbiturates



Compound No.	Ar.	Per cent inhibition* (Preincubation time in min.) 0 min.	10 min.	20 min.	30 min.
I		$58 \cdot 1 \pm 0 \cdot 2$	$58 \cdot 0 \pm 0 \cdot 2$	$58 \cdot 0 \pm 0 \cdot 4$	$58 \cdot 1 \pm 0 \cdot 1$
II		$70 \cdot 1 \pm 0 \cdot 8$	$70 \cdot 0 \pm 0 \cdot 5$	$70 \cdot 1 \pm 0 \cdot 3$	$70 \cdot 2 \pm 1 \cdot 4$
III		$72 \cdot 0 \pm 0 \cdot 5$	$72 \cdot 0 \pm 0 \cdot 4$	$72 \cdot 2 \pm 0 \cdot 3$	$72 \cdot 1 \pm 0 \cdot 9$
IV		$76 \cdot 0 \pm 1 \cdot 5$	$76 \cdot 1 \pm 0 \cdot 7$	$76 \cdot 1 \pm 0 \cdot 6$	$76 \cdot 0 \pm 0 \cdot 9$
V		$92 \cdot 3 \pm 0 \cdot 5$	$92 \cdot 2 \pm 0 \cdot 4$	$92 \cdot 1 \pm 0 \cdot 4$	$92 \cdot 2 \pm 0 \cdot 7$
VI		$70 \cdot 8 \pm 1 \cdot 5$	$70 \cdot 5 \pm 0 \cdot 9$	$70 \cdot 6 \pm 0 \cdot 3$	$70 \cdot 7 \pm 0 \cdot 6$
VII		$98 \cdot 1 \pm 1 \cdot 0$	$98 \cdot 2 \pm 0 \cdot 8$	$98 \cdot 2 \pm 0 \cdot 5$	$98 \cdot 3 \pm 0 \cdot 7$
VIII		$94 \cdot 7 \pm 0 \cdot 9$	$94 \cdot 8 \pm 1 \cdot 3$	$94 \cdot 7 \pm 0 \cdot 3$	$94 \cdot 8 \pm 0 \cdot 6$
IX		$92 \cdot 3 \pm 0 \cdot 4$	$92 \cdot 1 \pm 0 \cdot 8$	$92 \cdot 0 \pm 1 \cdot 0$	$92 \cdot 2 \pm 1 \cdot 2$

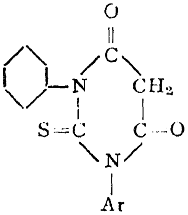
* Assay procedures and contents of the reaction mixture were as described in the text. Each experiment was done in triplicate and the values are the mean of three separate experiments with ± standard error of the mean. The compounds were used at a final concentration of 1×10^{-3} *M*.

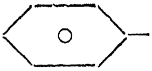
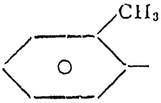
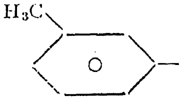
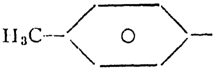
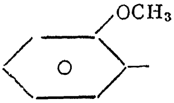
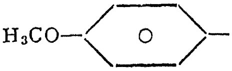
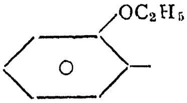
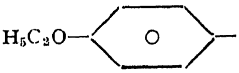
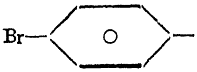
shifting of electron-donating groups from position 2 to 4 of the phenyl nucleus produced greater degree of protection, while the inhibition of succinate dehydrogenase activity was lowered. The partition coefficients of these compounds are also shown in Table V. These values could not be correlated to either the anticonvulsant activity or SDH inhibitory activity.

Some 1, 3-disubstituted thiobarbiturates have been reported to affect the succinoxidase activity of rat brain homogenate and the site of action of these

TABLE V

Anticonvulsant activity and partition coefficients of 1-aryl-3-cyclohexyl thiobarbiturates



Compound No.	Ar	Anti-convulsant activity[a] %	Mortality[b] %	Partition coefficients
I		10	70	7·40
II		40	30	6·14
III		40	60	7·33
IV		60	20	6·73
V		30	40	25·56
VI		50	30	19·75
VII		20	60	15·20
VIII		30	40	12·15
IX		20	70	28·41

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[a] Anticonvulsant activity was determined as described in the experimental section.

[b] Represents mortality in each group of animals administered pentylenetetrazol during 24 hr period.

ompounds has been demonstrated to be presumably at ıe complex I and complex II of the electron transport hain[7]. The present investigation was made in an ttempt to investigate the effect of 1-aryl-3-cyclohexyl- ıiobarbiturates on complex II of electron transport chain. In the present study the inhibitory action of these thiobarbiturates on complex II of E.T.C. can be ascribed to be due to the action of these compounds on succinate dehydrogenase, a constituent of complex II. However, these studies do not show a definite correlation between the anticonvulsant activity of these 1-aryl-3-cyclohexyl thiobarbiturates and their ability to inhibit succinate dehydrogenase of rat brain homogenate as a biochemical basis of their anticonvulsant activity.

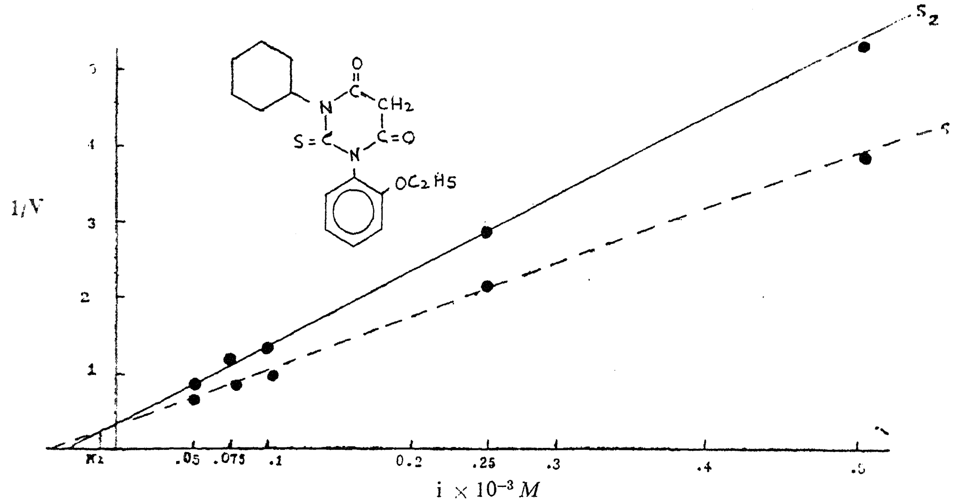

FIG 1. Kinetic study showing competitive inhibition of rat brain succinate dehydrogenase by 1-(2-ethoxyphenyl)-3-cyclohexyl-thiobarbiturate, 1/V represents reciprocal of the enzyme activity while (i) represents inhibitor concentrations. Final concentrations of the substrate were S_1 = ·005 *M*, S_2 = ·0025 *M*.

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1. Parmar, S. S., Joshi, P., Ali, B. and Cornatzer, W. E., *J. Pharm. Sci.*, 1974, **63**, 872.
2. Barthwal, J. P., Agarwal, V. K., Tandon, S. K., Dixit, K. S. and Parmar, S. S., *Ibid*, 1974, **62**, 613.
3. Parmar, S. S., Dwivedi, C., Ali, B. and Misra, R. S., *J. Med. Chem.*, 1972, **15**, 864.
4. Schroeter, P., *Acta Neuropathol.*, 1963, **2** (5), 470.
5. Chetverikota, E. P. *Voprosy Med. Khim.*, 1958, **4**, 131.
6. Zimmermenn, H. and Platt, D., *Frankfurter, Z. Pathol.*, 1962, **71**, 453.
7. Pandey, B. R., *Ph.D. Thesis* (Chemistry), Lucknow University, 1975.
8. Kumar, S., *Ph.D. Thesis* (Biochemistry), Lucknow University, 1976.
9. Nagar, S., Singh, H. H., Sinha, J. N. and Parmar S. S., *J. Med. Chem.*, 1973, **16**, 178.
10. Slater, E. C. and Bonner, W. D., *Biochem. J.*, 1952, **52**, 185.
11. Parmar, S. S., Dwivedi, C., Chaudhary, A. and Gupta, T. K., *J. Med. Chem.*, 1972, **15**, 99.
12. Lineweaver, H. and Burk, D., *J. Am. Chem. Soc.*, 1934, **56**, 658.
13. Dixon, M., *Biochem. J.*, 1953, **55**, 170.

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INFLUENCE OF SEED BACTERIZATION ON NODULATION, YIELD AND AMINO ACID COMPOSITION OF SEED PROTEIN OF *CICER ARIETINUM* VAR. TYPE-1

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ABSTRACT

The role of different strains of *Rhizobium* sp. (gram) H_{44}, H_{45}, *Beijerinckia indica* J_3 and *Azotobacter chroococcum* B_4, on nodulation, seed yield and amino acid compostion of seed protein of *Cicer arietinum* var. type-1 were assessed under the field conditions, following the randomized block design with a basal dressing of 20 kg N and 50 kg P_2O_5/ha. Simultaneous inoculation with *Azotobacter* and any of the strains of *Rhizobium* proved better than *Rhizobium* or *Azotobacter* or *Beijerinckia* or *Rhizobium*+*Bejerinckia* towards the yield, Out of the 16 amino acids detected in the seeds, aspartic acid, glutamic acid, isoleucine and phenylalanine were maximum due to strain H_{45}; threonine, glycine and valine due to strains $H_{45} + J_3$; tyrosine and histidine due to strains $H_{45} + B_4$; and alanine due to strain B_4. Aspartic and glutamic acids tended to decrease due to simultaneous inoculation with *Rhizobium* and *Azotobacter* when compared with *Rhizobium* or *Azotobacter* alone; whereas some of the amino acids showed an increasing trend. *Azotobacter* when used with *Rhizobium* caused the formation of fewer but healthier nodules and perhaps the better utilization of the symbiotically fixed nitrogen by the crop.

YIELD increases were reported by Sheloumova[8] (1941) with simultaneous treatment of some leguminous seeds with azotobacterin and root nodule bacteria specific for them. Synergistic effect of gram *Rhizobium* H_{44} and *Azotobacter chroococcum* B_4 was recorded by Rawat and Sanoria[6] (1976) on the grain yield. Employing *Rhizobium*, *Azotobacter* and *Beijerinckia* either alone or in combinations of the later two with *Rhizobium*, experiments were tried following the same design and technique. The primary objective was to assess the effect of inoculation on both the quality and quantity of seeds.

MATERIAL AND METHODS

Gram rhizobia, *Beijerinckia indica* and *Azotobacter chroococcum* used in this study are isolates from Sahapura block soils of Jabalpur, kitchen garden soil of Jabalpur and B.H.U. farm soil of Varanasi respectively. *Rhizobium* isolates were serologically distinct (Vaishya[9], 1971) while *Beijerinckia* and *Azotobacter* were morphologically different. *Beijerinckia* had the capability to grow at pH 3·0 and it produced abundant slime. *Rhizobium* was grown on yeast extract mannitol agar medium (Vincent[10], 1970) slants and *Azotobacter* and *Beijerinckia* were grown on Burk's medium (Rubenchik[7], 1963). Preparation of cultures and the method of seed inoculation was the same as described by Rawat and Sanoria[6] (1976).

The plants were uprooted for nodule study 70 days after sowing. Crude protein in seeds was determined by Kjeldahl method and the aminо acids were estimated with Hitachi-Perkin Elmer KLB-3B automatic amino acid analyser.

RESULTS AND DISCUSSION

Nodule weight, instead of the nodule number, is considered to be a better criterion for evaluating the efficiency of rhizobia. Extent of symbiotic nitrogen fixation thus depends upon the nodular mass. Sometimes irregularities do appear because of the formation of nodules by inefficient but active strains. In such cases the nodules will either be deprived of or remain poor in leghemoglobin, the real compound of symbiotic origin (Burris[2], 1974). Looking to the data on nodulation, yield and crude protein content of the seeds (Table I), it appears that seed bacterization with either *Azotobacter* or *Beijerinckia*, stimulates slightly the native rhizobia which in turn causes somewhat better nodulation over the control. Both the strains of gram *Rhizobium* sp. brought about greater nodulation and increased the nodular mass but the grain yield was not significantly improved over the control. Of course, *Rh.* H_{44} promoted the vegetative growth. Similarly, *Rh.* H_{45} with *Beijerinckia* was beneficial for the straw yield. When *Rhizobium* strains were used along with *Azotobacter* or *Beijerinckia*, there was reduction in the nodule numbers in comparison to *Rhizobium* alone. In contrast to *Azotobacter*, *Beijerinckia* inoculation, along with *Rhizobium*, decreased the nodular mass.

From the point of view of crop yield, treatment *Rh.* H_{44} + *Azotobacter* stood first and next to it was the treatment, *Rh.* H_{45} + *Azotobacter*. Rao Kumar and Patil[5] (1976) reported better yields of soybean due to inoculation with *Rhizobium* and *Azotobacter* whereas Rajani Apte and Iswaran[4] (1974) obtained good yields of soybean with *Rhizobium* and *Beijerinckia*. By the use of *Azotobacter* with *Rh.* H_{44} or *Rh.* H_{45}, although there was reduction in the number of nodules yet, the weight of individual nodules had increased. This observation supports the hypothesis that the total nodule weight (instead of nodule number) should be considered as a criterion in legume *Rhizobium* symbiosis. In the treatment of *Rh.* H_{45} the weight of nodules

TABLE I

Nodulation, characteristics, yield and crude protein content of Bengal gram

Treatments	Total No. of nodules per plant	Oven dry weight of nodules per plant	Straw yield (Q/ha)	Grain yield (Q/ha)	Seed crude protein (%)
1. Control	3·25	14·25	34·75	24·58	24·71
2. *Rhizobium* sp. H_{44}	56·75**	160·25**	45·37*	26·50	25·65
3. *Rhizobium* sp. H_{45}	31·00**	133·00**	41·50	25·50	24·99
4. *Beijerinckia indica* J_3	17·00	36·50	40·37	24·00	25·25
5. *Azotobacter chroococcum* B_4	11·00	27·50	40·50	24·58	24·26
6. *Rh.* sp. H_{44} + *Beij. indica* J_3	33·00**	96·50**	40·75	23·58	23·34
7. *Rh.* sp. H_{44} + *A. chroococcum* B_4	24·75**	202·25**	47·75**	29·50**	25·35
8. *Rh.* sp. H_{45} + *Beij. indica* J_3	23·25*	94·75*	46·37**	24·75	23·74
9. *Rh.* sp. H_{45} + *A. chroococcum* B_4	21·75*	104·00**	47·50**	28·25*	24·30
C.D. at 5%	17·95	60·26	8·15	3·11	N.S.
1%	24·38	81·67	11·04	4·17	N.S.

* Significant at 5% level of significance. ** Significant at 1% level of significance.

plant is 133 mg whereas in the treatment *Rh.* H_{45} + *Azotobacter* it is 104 mg. That the yield was increased by the latter treatment, and not in the former, warrants for an alternative explanation.

In the absence of data on leghemoglobin content which could have been taken as the parameter for the amount of nitrogen fixed symbiotically, one has to consider the mode of utilization of the fixed nitrogen. Referring to data in Table II on amino acid composition of seeds, some explanation is possible. In the control, the amino acids serine, proline, leucine, lysine and arginine are in the highest amount. *Rh.* H_{45} gave maximum amount of aspartic acid, glutamic acid, isoleucine, norleucine and phenylalanine while in combination with *Beijerinckia* it resulted in the highest amount of threonine, glycine and valine. *Azotobacter* alone increased alanine. Linta[3] (1963) reported an increase in the proportion of protein, nitrogen and amino acids in pea and vetch when inoculated with active strains of rhizobia.

Results of Table I show the synergistic behaviour of *Azotobacter* and the *Rhizobium* strains on crop yield. Similar behaviour of the combined culture is noticeable on the amino acid composition of seeds. By using *Rh.* H_{44} and *Azotobacter* there has been an increase in some amino acids like serine, tyrosine and lysine in comparison with the use of *Rh.* H_{44} or *Azotobacter* alone. Similarly, increases in glycine, valine, tyrosine and histidine were due to *Rh.* H_{45} + *Azotobacter* as compared with the individual cultures. Decrease in aspartic acid and glutamic acid due to *Rh.* H_{44} + *Azotobacter* or *Rh.* H_{45} + *Azotobacter* appears to be more significant in this context because these acidic amino acids are formed first in the process of symbiotic N-fixation (Bala Ravi[1], 1975). Decrease in their amount suggests indirectly their conversion into other amino acids. From the discussion it appears that *Azotobacter* when used along with *Rhizobium*, besides causing the formation of fewer and healthier nodules, helps in the better utilization of symbiotically fixed nitrogen. The other possible beneficial effects of *Azotobacter* in combination with *Rhizobium* have been discussed by Rawat and Sanoria[6] (1976).

TABLE II.

Amino acids expressed in g/100 g protein from seeds of different treatments

Amino acid	Control	*Rhizobium* sp. H_{44}	*Rhizobium* sp. H_{45}	*Beijerinckia indica* J_3	*Azotobacter chroococcum* B_4	*Rh.* sp. H_{44} +*Beij. indica* J_3	*Rh.* sp. H_{44}+*A. chroococcum* B^4	*Rh.* sp. H_{45} +*Beij. indica* J_3	*Rh.* sp. H_{45}+*A. chroococcum* B_4
Aspartic acid	9·69	10·32	11·99*	9·13	10·73	10·89	9·89	10·83	10·70
Glutamic acid	12·71	13·64	14·53*	12·93	14·01	13·93	12·31	13·81	13·79
Hydroxy proline	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Serine	4·74*	3·23	3·45	4·36	3·87	3·56	4·52	3·50	3·40
Threonine	2·49	2·60	2·29	2·53	3·14	3·20	2·79	3·41*	3·17
Proline	4·61*	4·45	3·88	4·32	4·03	3·97	4·53	3·99	4·00
Alanine	3·31	3·35	3·97	3·34	4·30*	4·11	4·01	4·07	4·21
Glycine	5·72	6·67	5·30	5·80	7·21	7·59	6·33	8·01*	7·70
Valine	4·32	4·48	4·53	4·28	5·13	6·09	4·89	6·71*	6·21
Cystine	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Methionine	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Isoleucine	3·78	3·62	4·01*	3·65	3·33	3·62	3·69	3·67	3·71
Leucine	6·95*	6·83	6·79	5·98	6·91	6·73	6·82	6·81	6·81
Norleucine	2·51	2·49	2·53*	2·50	2·51	2·53*	2·50	2·50	2·51
Tyrosine	2·29	2·13	2·04	2·39	2·61	3·92	3·97	3·99	4·01*
Phenylalanine	4·87	3·90	5·64*	4·63	5·07	5·53	5·03	5·20	5·61
Lysine	6·21*	5·01	5·65	6·00	5·16	5·27	5·81	5·18	5·30
Histidine	1·31	1·27	1·09	1·47	2·27	2·32	1·92	2·41	2·71
Ammonia	0·63	0·27	0·52	0·59	0·62	0·67	0·82	0·59	0·62
Tryptophan	ND	ND	ND	ND	ND	ND	ND	ND	ND
Arginine	12·67*	7·87	7·59	10·89	8·13	7·89	7·93	7·50	7·77
TOTAL	88·81	82·13	85·80	84·79	90·03	91·82	87·76	92·18	92·23

Results are expressed:

1. On moisture free basis.
2. Protein conversion factor is used 6·25.

NQ. Not quantitated in acid hydrolysate.
ND. Not detected in acid hydrolysate.
* Maximum among all the treatments.

ACKNOWLEDGEMENT

We are thankful to Dr. Sant Singh, Head of the Department of Soil Science and Agricultural Chemistry, B.H.U., for providing facilities. Our special thanks are due to Dr. D. Raj, Head of the Biochemistry Department, Tamil Nadu Agricultural University, Coimbatore, for getting the seeds analysed for amino acids. One of us (A. K. R.) is indebted to U.G.C. for providing financial help during this study.

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1. Bala Ravi, S., *Science Today*, 1975, **10** (5), 15.
2. Burris, R. H., *Plant Physiol.*, 1974, **53**, 443.
3. Linta, *Agrokem. Tala*, 1963, **12**, 647.
4. Rajani Apte and Iswaran, V., *Proc. of the Ind. Nat. Sci. Acad.*, 1974, Part B, **40** (5), 482.
5. Rao Kumar, J. V. D. K. and Patil, B. D., *Curr. Sci.*, 1976, **45** (14), 523.
6. Rawat, A. K. and Sanoria, C. L., *Ibid.*, 1976, **45** (18), 665.
7. Rubenchik, L. I., *Azotobacter and Its Use in Agriculture*, Oldbourne Press, 1963, p. 2.
8. Sheloumova, A. M., *Mikrobiologiya*, 1941, **10** (1).
9. Vaishya, U., *M.Sc. Thesis*, J.N.K.V.V., Jabalpur, 1971, p. 14.
10. Vincent, J. M., *A Manual for the Practical Study of Root Nodule Bacteria*, Blackwell Scientific Publications, Oxford and Edinburgh, 1970.

LETTERS TO THE EDITOR

MEASUREMENTS OF L-SHELL PHOTOELECTRIC CROSS SECTIONS IN HEAVY ELEMENTS AT 60 keV

In order to fill the void in the existing data[1] on inner shell photoelectric cross sections, measurements of L shell cross sections at 60 keV in elements W, Hg, Tl, Pb, Bi and U have been made. The method used is similar to the methods used earlier [2,3] and consists of measuring the intensity of emitted L-shell fluorescent X-rays with a calibrated proportional counter spectrometer when a target is irradiated with known flux of 60 keV photons obtained from Am^{241}. As discussed in an earlier communication[2], the other low energy photons, *i.e.*, conversion L X-rays in N_p and 26 keV gamma rays emitted from the source were filtered by a suitable graded absorber. As no other experimental data are available[1], the results have been compared with the recent theoretical calculations of Scofield[4] in Table I. The experimental result are found to agree with theory within errors which are somewhat smaller than those in the earlier measurements[2] at 145 and 279 keV. In the present measurements, in contrast to the earlier measurements, the incident photons are unable to excite the K-shell electrons of the target element and thus there is no contribution to the L shell fluorescent radiation due to the filling of K-shell vacancies by the jump of L-shell electrons. The term

$$\sigma_K f_{KL} \frac{\bar{W}_{KL}}{\bar{W}_L}$$

in equations 1 and 3 of ref. 2 is, therefore, not needed for the evaluation of the present cross sections. Thus the overall error in the final results is reduced.

TABLE I

Comparison of the present measurements of L-shell photoelectric cross sections at 60 keV *with theoretical calculations of Scofield*

Element	L-shell photoelectric cross sections at 60 keV	
	Present measurements (b/atom)	Theoretical values (b/atom)
W	770 ± 60	760
Hg	1100 ± 80	1050
Tl	1150 ± 80	1110
Pb	1250 ± 90	1170
Bi	1280 ± 90	1230
U	2040 ± 150	1900

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---

1. Hubbell, H. J. and Veigele, Wm. J., NBS Technical Note No. 901, April 1976.
2. Singh, M. J. and Sood, B. S., *Nuovo Cimento*, 1972, **8B**, 261.
3. Allawadhi, K. L. and Sood, B. S., *Phys. Rev.*, 1975, **A11**, 1928.
4. Scofield, J. H., *Lawrence Livermore Laboratory Report*, 1973, UCRL 51326.

ANNEALING CORRECTION TO THE FISSION TRACK AGES OF PHLOGOPITES

Thermal events occurring in the geological history of a region affect the fission track ages of minerals. Thus a correction to these ages must be applied which in the case of phlogopites (Borra Mines, Visakhapatnam District) has been estimated to be 7%.

In order to apply correction for thermally lowered fission track ages, annealing experiments have been performed by a number of workers[1,2]. The radiation damage in minerals tends to heal during short time high temperature event or a long time anneal at a slightly elevated temperature.

In our present study the phlogopite samples were irradiated with a thermal neutron dose of $1 \cdot 2 \times 10^{16}$ (nvt) in CIRUS reactor at B.A.R.C., Trombay. These irradiated samples of known induced track density were heated in a muffle furnace at different constant temperatures for one hour and the reduction in track density and track length with temperature have been observed. The results are summarized in Table I.

It is evident from Table I that the induced fission tracks in phlogopite do not disappear completely for one hour heating even at 800° C. Thus for complete removal of tracks, intense heating is required. Figure 1 shows that the reduction in track density at all temperatures lags behind the reduction in track length

TABLE I

Annealing data of phlogopites
Heating time 1 hr

Temperature °C	Percentage reduction track length	Percentage reduction track density
200	3·3	Negligible
300	10·3	8·2
400	17·0	10·7
500	27·3	20·6
600	32·8	30·7
700	50·3	49·0
800	57·4	55·0

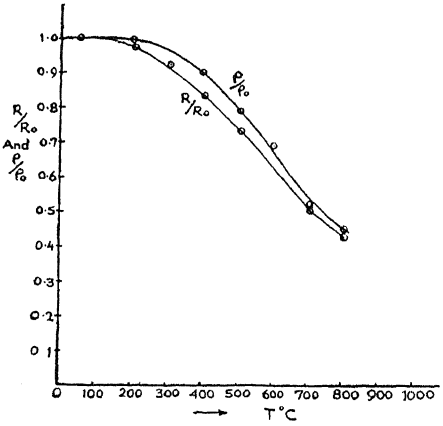
The fission track ages of phlogopites and muscovites have been determined. The experimental details are the same as reported elsewhere[1,3]. The fission track ages are calculated by using the simplified version of the formula[4]

$$T = 6\cdot57 \times 10^{9} \ln [1 + 9\cdot25 \times 10^{-18}\, \rho_s/\rho_i \times \phi]$$

where ρ_s and ρ_i are the fossil and induced track densities respectively and ϕ is the total thermal neutron dose with which the samples have been irradiated. The fission track ages are given in Table II. The mean age for phlogopites is 491 ± 16 m.y. and that for muscovites is 621 ± 142 m.y. The errors in the results are statistical counting errors. These ages correspond to the last orogenic metamorphic cycle in the geological history of the region.

A linear relation has been observed between track length reduction and track density reduction (Fig. 2). In phlogopite, the mean range of fossil tracks is less than the mean range of induced tracks by 10%. This reduction in track length corresponds to 7% decrease in fossil track density. Hence the correction to fission track ages of phlogopites is also of the same order.

Our age results show an excellent agreement with fission track ages determined by Kere[5] for Gudur biotites and those of Nand Lal *et al.*[6], for apatites of the same region. Thus from the comparative studies on fission track ages of apatite, phlogopite and biotite we conclude that fairly stable thermal conditions have



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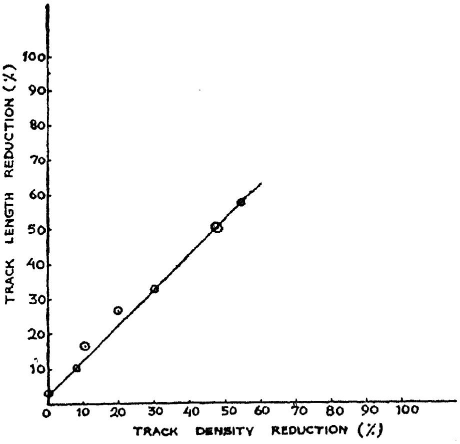

2

FIGS. 1–2. Fig. 1. Relation between mean range and track density of induced fission tracks during annealing. Fig. 2. Percentage reduction in track density *versus* percentage reduction in track length.

TABLE II

Fission track ages of some muscovites and phlogopites of Andhra Pradesh

Total neutron dose $\phi = 1\cdot2 \times 10^{16}$ (nvt)

Location	Mineral	No. of samples studied	Track density ρ_s/ρ_i	Mean f.t. age (m.y.) T	U. Conc. (atom/atom) $\times 10^{-10}$
Borra Mines	Phlogopite	5	0·70	491±16	79·0
Seetha Rama Mines	Muscovite	5	0·90	621±142	1·2

been prevailing in the East Coast region since the last metamorphic cycle known as Indian Ocean Cycle.

In comparison with our previous studies on the annealing behaviour of muscovite[7] and biotite[8], it has been observed that the correction for loss of tracks by annealing to the fission track ages of phlogopites is slightly less than that for muscovite and biotite.

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---

1. Fleischer, R. L., Price, P. B. and Walker, R. M., *Nuclear Tracks in Solids*, Univ. of California Press, California, 1975.
2. Nagpaul, K. K., Mehta, P. P. and Gupta, M. L., *Pure Appl. Geophys.*, 1974, **112**, 131.
3. Virk, H. S. and Singh, Surinder, *Min. J. Japan*, 1977, **8**, 263.
4. — and Koul, S. L., *Ind. J. Pure Appl. Phys.*, 1974, **12**, 850.
5. Kere, S. S., *Curr. Sci.*, 1966, **35**, 509.
6. Nand Lal, Nagpaul, K. K. and Nagpaul, M. K., *Ibid.*, 1975, **44**, 411.
7. Virk, H. S. and Koul, S. L., *Ibid.*, 1975, **44**, 341.
8. — and Singh, Surinder, *Ind. J. Pure Appl. Phys.*, 1976, **14**, 421.

A NEW INDICATOR IN THE SEQUENTIAL COMPLEXOMETRIC ANALYSIS OF COPPER AND NICKEL

A NUMBER of reliable analytical procedures for the determination of Cu (II) and Ni (II) are available. There is however no simple volumetric method available for the determination of these metals when present together.

α-mercaptopropionic acid (MPA) forms a violet complex with Cu(II) in the pH range 2·5 and 8[1]. In this paper the application of copper–MPA complex as an indicator in the sequential complexometric estimation of copper and nickel in Cu–Ni alloys is described.

Experimental

Reagent: MPA of analytical grade was used to prepare 1% solution in double distilled water. Stock solutions of copper and nickel (0·1 M) were prepared and analysed by standard methods[2].

Procedure

Aliquots of Cu(II) and Ni(II) solutions containing 3 to 20 mg of the metals were taken in a conical flask. The pH was adjusted between 3 and 4 with phthalate or acetate buffer and two drops of the reagent were added when an intense violet colouration was obtained. The mixture was then titrated against a solution o EDTA of known strength till the colour changed from violet to bluish green. The burette reading was th total reading for Cu(II) and Ni(II).

Again the same aliquots of Cu(II) and Ni(II) solutions were taken in a separate conical flask and th pH of the mixture was adjusted to between 3 and 4 as before. To this was added about two grams o potassium citrate and about two drops of the reagent. The mixture was titrated with the same solution of EDTA till the colour changed from violet to bluish green. The burette reading is for copper only.

Analysis of Copper-Nickel Alloys

In the case of the analysis of copper-nickel alloys known weight of the given alloy was dissolved in nitric acid and the solution prepared in the usual way. The aliquots of the solution can be used for the analysis of copper and nickel as above (Table I).

TABLE I

Analysis of some standard Cu-Ni alloys with MPA as an indicator

Sr. No.	Percentage of metals expected		Percentage of metals found	
	Cu	Ni	Cu	Ni
1.	87·2	11·3	87·0	11·15
2.	85·5	13·5	85·37	13·4
3.	81·0	17·0	81·12	16·85
4.	77·0	21·0	76·82	20·80

It was found that ions like $S_2O_3^{2-}$, NO_2^-, I^-, $Cr_2O_7^{2-}$, MnO_4^- interfere seriously and should be absent, however, ions like Cl^-, Br^-, $B_4O_7^{2-}$ could be tolerated ten times in excess, while ions like SO_3^{2-} and ClO_4^- could be tolerated only five times in excess.

Our sincere thanks are due to Prof. G. S. Deshmukh for the helpful discussions.

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---

1. Klotz, I. M., Czerischski, G. H. and Fiess, H. A., *J. Am. Chem. Soc.*, 1958, **80**, 2920.
2. Vogel, A. I., *Quantitative Inorganic Analysis*, Longmans Green and Co. Ltd., London, 3rd Edn., 1975.

BROMATOMETRIC DETERMINATION OF PHENOLS AND PHENOLIC CARBONYL COMPOUNDS

Koppeschaar's[1] bromatometric method is extensively used for the determination of phenols and related compounds. In practice two procedures are used which differ significantly in the magnitude of the excess of the brominating mixture added. The complications which may occur in this method are discussed elsewhere[3-9].

Neelakantam and Viswanath[10] investigated the bromination of the phenolic ketone and resacetophenone, by the excess method and found that it did not stop at either the theoretical dibromination or even the tribromination stage. Direct titration with the brominating mixture using methyl orange as indicator was employed by Ramanujam[11] for the determination of the ketone and also for the analysis of the copper complex[12], and by Raja Reddy for phenol[13]. The results corresponded to di- and monobromination respectively for the ketone and its monomethyl ether. This method appears to be better suited for the volumetric analysis of metallic complexes with *o*-hydroxy carbonyl compounds.

In the present investigation the determination of phenols and phenolic carbonyl compounds was carried out following the procedure described by Raja Reddy[13] at 30–35° C.

The results are summarised below:

1. Phenol[3,6,] undergoes tribromination.
2. Salicylic acid undergoes dibromination without decarboxylation. In the excess method, it undergoes tribromination and decarboxylation above 20° C[2,3,6].
3. *p*-Cresotic acid (6-hydroxy-3-methyl benzoic acid) undergoes dibromination and decarboxylation.
4. Thymol[6] (6-isopropyl-*m*-cresol) undergoes dibromination.
5. Vanillin[3] undergoes dibromination with the elimiantion of the aldehyde group whereas it undergoes monobromination at 0° C.
6. Pyrogallol undergoes dibromination and gallic acid undergoes monobromination only.
7. *p*-Hydroxyacetophenone undergoes dibromination.
8. *β*-Naphthol[6] undergoes monobromination and 2-hydroxy-3-naphthoic acid, dibromination and decarboxylation.

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---

1. Koppeschaar, *Ziet Anal Chem.*, 1876, **15**, 234.
2. Allen, *Commercial Organic Analysis*, IV edn., Blackiston's Son and Co. (Philadelphia), 1910, **3**, 480.
3. Francis, A. W. and Hill, A. J., *J. Am. Chem. Soc.*, 1924, **46**, 2498.
4. —, — and Johnstone, J., *Ibid.*, 1925, **47**, 2213.
5. —, *Ibid.*, 1926, **48**, 1631.
6. Day, A. R. and Taggart, W. T., *Ind. Eng. Chem.*, 1928, **20**, 545.
7. Sprung, M. M., *Ibid.* (Anal. Ed.), 1941, **13**, 35.
8. Francis, A. W. and Hill, A. J., *Ibid.*, 1941, **13**, 357.
9. Ruderman, I. W., *Ibid.*, 1946, **18**, 753.
10. Neelakantam, K. and Viswanath, G., *Proc. Ind. Acad. Sci.*, 1952, **35A**, 72.
11. Ramanujam, V. V., *J. Sci. Ind. Res.*, 1955, **14 B** (11), 564.
12. —, *Ibid.*, 1956, **15 B** (4), 212.
13. Raja Reddy, G., *Curr. Sci.*, 1966, **35**, 172.

SOME OBSERVATIONS ON REGULAR STRUCTURES IN PROTEINS

Ever since Pauling discovered *α*-helix[1], it was expected that the globular protein molecules would contain high percentage of *α*-helices because of the great stability of this conformation. But when crystal structures of many proteins were solved, it was apparent that it was not quite so. Many of the globular proteins were found to contain *β*-sheets too, which are the other predicted low-energy conformation of polypeptide chains[2]. Apart from these regular structures of *α*-helices and *β*-sheets, considerable portion of the polypeptide chains in globular proteins contain non-regular conformations, where the successive units do not have repeating conformational features.

The purpose of this note is to attract attention to an interesting feature of protein folding. In almost all the proteins roughly half the number of the residues are involved in either of the two regular structures, *viz.*, *α*-helices and *β*-sheets. Figure 1 shows the plot of a number of residues involved in these two regular conformations, against the total number of residues, for various protein molecules (the data have been compiled from refs. 3–5). The abbreviations and the corresponding common names of the proteins shown in this figure are: INS—Insulin; PTI—Pancreatic trypsin inhibitor; CYT—Cytochrome; MGN—myoglobin; CPM—carp muscle calcium binding protein; LYZ—Lysozyme; TPG—Trypsinogen; CNA—Concanavalin A; EST—Elastase; SBT—

Subtilisin; CPA—Carboxypeptidase A: LDH—Lactate dehydrogenase.

It is seen from Fig. 1 that about 45% of the total number of residues in a protein are having regular conformations of α-helices or β-strands. A straight line corresponding to this value of 45% has been drawn in the figure to show how closely the rule is followed. A tentative explanation for this remarkable tendency in the folding of protein molecules may be given as follows: The regular conformations of α-helices and β-strands have considerably low energy-values[6], enhancing their probability of occurrence in protein molecules. But, while forming a globular shape in the acqueous medium the residues have to take up conformations other than α-helices and β-strands and remarkably enough, the percentage composition of these non-regular conformations is almost a constant (55%).

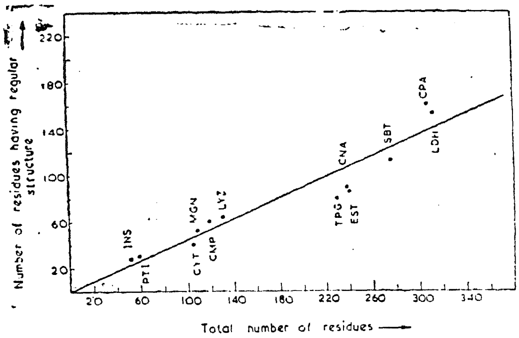

FIG. 1. The plot of number of residues in regular conformations *versus* the total number of residues, in various proteins. For details see text.

Of course there are a few exceptions, like myoglobin[7] which has about 80% of the residues forming a special nonpolar packet for the heme group and triose phosphate isomerase which has a $(\beta\alpha)_8$ barrel[8] consisting of 55% of α-helical residues and 22% of β-strands (a total of about 80% of residues in regular conformations). Further studies in these lines are in progress.

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---

1. Pauling, L., Corey, R. B. and Branson, H. R., *Proc. Natl. Acad. Sci.*, U.S., 1951, **37**, 205.
2. — and —, *Ibid.*, 1951, **37**, 729.
3. Lim, G. I., *J. Mol. Biol.*, 1974, **88**, 873.
4. Nagano, K., *Ibid.*, 1973, **75**, 401.
5. —, *Ibid.*, 1973, **84**, 337.
6. Ramachandran, G. N. and Sasisekharan, V., *Adv. Protein Chem.*, 1968, **23**, 283.
7. Kendrew, J. C., Dickerson, R. E., Strandsberg, B. E., Hart, R. G. and Davies, D. R., *Nature*, 1960, **185**, 422.
8. Banner, D. W., Bloomer, A. C., Petsko, G. A., Phillips, D. C., Pogson, C. I., Wilson, I. A., Corran, P. H., Furth, A. J., Milman, J. D., Offord, R. E., Priddle, J. D. and Waley, S. G., *Ibid.*, 1975, **255**, 609.

SOME REACTIONS OF 3, 4-DICHLOROCOUMARINS

IN an earlier communication, we have described the syntheses of some 3, 4-dichlorocoumarins[1] by the reaction of hexachloropropene with phenolic compounds.

The present work reports some of the reactions of these halogenated coumarins.

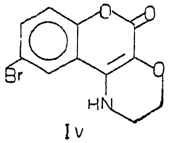
The condensation of 6, 7-dimethyl-3, 4-dichlorocoumarin[1] (I) was effected with piperidine, morpholine, diethylamine and N-methylpiperizine by boiling them in alcoholic solution to yield the respective 4-amino derivatives (m.p. 174°; 167–168°; 145° and 152–153° respectively). The latter were tested at the Central Drug Research Institute, Lucknow, but none of them showed any appreciable antibacterial activity.

Also, (I) on condensation with ethanolamine in refluxing methanol gave 3-chloro-4-(2-hydroxyethylamino)-6, 7-dimethyl-coumarin as a colourless crystalline solid, m.p. 212–214° (yield 50%). Attempts to cyclise it to an oxazine derivative were futile. However, 6-bromo-3, 4-dichlorocoumarin[2] (II) with ethanolamine gave 3-chloro-4-(2-hydroxyethylamino)-6-bromocoumarin (III), (m.p. 198–199°, yield 40%), which was cyclised in the presence of sodium hydride to yield 9-bromo-3, 4-dihydro [1]-benzopyrano-[3, 4-*b*] [1, 4] oxazin-5-[1H]-one (IV), crystallised from acetic acid in pale brown plates, m.p. 295–296°.

The coumarin (I) was also condensed with *o*-phenylenediamine in N-methylpyrrolidone to yield 3-chloro-4-(*o*-amino-anilino)-6, 7-dimethylcoumarin (V) (m.p. 213–215°) which was cyclised by heating the latter with pyridine and MnO_2 to give 2, 3-dimethyl-6H-[1] benzopyrano (3, 4-*b*) quinoxalin-6-one (VI) crystallised from glacial acetic acid in yellow plates, m.p. 265°.

It was observed that dichlorocoumarins having groups with -I effect such as chlroine, bromine, etc., in the benzene ring reacted with Grignard reagents easily, whereas electron donating substituents present in the dichlorocoumarin derivatives hindered the formation of 3-chloroflavones. Thus, it was observed that (I) did not react with phenyl magnesium bromide even under different experimental conditions. However, (II) reacted with this reagent to afford 3-chloro-6-bromoflavone in 40% yield. The structure (VII) assigned toit was consistent with spectral data showing a keto band in the i.r. at 1680 cm^{-1} and M$^+$ = 335.

Wihle working on 3-chloroflavones, our attention was drawn to a recent publication by Gerg *et al.*,[3] who have described the synthesis of 3-chloroflavones which are the key intermediates in the preparation of some physiologically active compounds.[c] Some 3-chloroflavone derivatives synthesised earlier by us pertaining to our work are described here.



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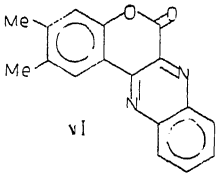

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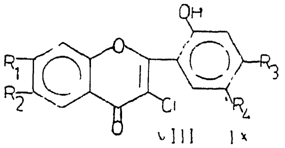

VIII IX

VIII: $R_1 = R_2 = R_3 = R_4 =$ Me.
IX: $R_1 = R_3 =$ H; $R_2 = R_4 =$ Br.

3, 4-Dimethylphenol (0·01 mole), anhydrous aluminium chloride (0·011 mole) and hexachloropropene (0·01 mole) in carbon disulphide were reacted in the usual manner. The chloroaluminium salt (prepared from 0·01 mole, 3, 4-dimethyl phenol and 0·011 mole anhydrous aluminium chloride in carbon disulphide) was added to the above solution and the mixture stirred for 24 hr. The solvent was removed under reduced pressure and the dark tarry mass was decomposed with ice and dilute sulphuric acid. The brown residue was filtered and triturated with methanol and again filtered. The filtrate on concentration gave a colourless compound which on repeated crystallisations from benzene-petrol ether (40-60°) yielded the pure sample which was found to be 6, 7-dimethyl-3, 4-dichlorocoumarin (1), (confirmed by mixed m.p. with authentic sample). The residue on repeated crystallisations from benzene-acetone gave a pink compound (TLC single spot) which showed molecular ion peak M$^+$ at 329 indicating the molecular formula to be $C_{19}H_{17}O_3Cl$. The i.r. spectrum showed a band at 1680 cm^{-1} (⟩ C = 0) in addition to the usual aromatic bands at 1600, 1500 and 900 cm^{-1}. From the above data, the compound was assigned the structure 3-chloro-6, 7, 4′, 5′-tetramethyl-2′-hydroxyflavone (VIII).

Similarly, *p*-bromophenol gave 6-bromo-3, 4-dichlorocoumarin (II) (confirmed by mixed m.p. with the authentic sample) and 3-chloro-6, 5′-dibromo-2′-hydroxyflavone (IX). They were separated by the fractional crystallisations. The i.r. spectrum of the flavone showned the ketonic band at 1680 cm^{-1} while the mass spectrum showed molecular ion peak M$^+$ and 431, confirming the assigned structure.

All the compounds gave satisfactory elemental analyses.

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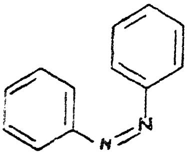
1. Merchant, J. R. and Rege, D. V., *Indian J. Chem.*, 1971, 9 (12), 1419.
2. Newman, M. S. and Schiff, S., *J. Am. Chem. Soc.*, 1959, **81**, 2266.
3. Gerg, C. P., Rastogi, M. K. and Kapoor, R. P., *Indian J. Chem.*, 1976, **14 B**, 470.

PHOTOCYCLIZATION OF 2-PHENYLAZOPYRIDINE : FORMATION OF 4-PYRIDO [*c*] CINNOLINE

PHOTOCHEMICAL cyclodehydrogenation of azobenzene (I) to benzo [*c*] cinnoline (II) occurs on irradiation of azobenzene in presence of proton acids[1] or in presence of Lewis acids[2,3]. As this reaction is of technological importance in connection with the photochemical fading of azo-dyes[4], it was thought of interest to examine similar reactions in the case of 2-phenylazopyridine (III). This communication describes the results of the irradiations of 2-phenylazopyridine under different conditions. The formation of the photocyclized product, 4-pyrido [*c*] cinnoline (IV), accounts for the fading observed in all these cases.

2-Phenylazopyridine was prepared by the condensation of 2-aminopyridine with freshly sublimed nitrosobenzene in presence of pyridine and sodium hydroxide[5].

When a very dilute solution of 2-phenylazopyridine (0·0001 M) in petroleum ether was exposed to sunlight, a gradual bleaching of the colour of the solution from deep red to pale yellow was observed. The reaction was followed spectrophotometrically by the reduction in the intensity of the peak at 450 nm and appearance of a new peak around 380 nm characteristic of the cyclized product[1]. Bleaching occurred more rapidly when an ethanolic sulphuric acid solution of 2-phenylazopyridine was kept exposed to light.



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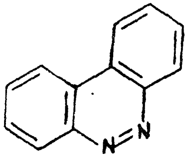
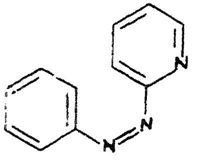


II



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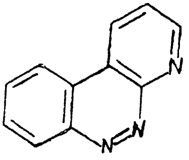

IV

A solution of 2-phenylazopyridine (500 mg) in ethanol (500 ml) containing concentrated sulphuric acid (50 ml) was irradiated in an Engelhard-Hanovia one-litre photochemical reactor with a Philips HPK 125 W high-pressure mercury-quartz lamp for 20 hr. Working-up and chromatography of the reaction mixture on a neutral alumina column afforded a greenish yellow fluorescent solid (120 mg), m.p. 193°, identified as 4-pyrido [*c*] cinnoline from elemental analysis and spectra.

4-Pyrido [*c*] cinnoline was also obtained by the irradiation of 2-phenylazopyridine in presence of anhydrous aluminium chloride in dichloromethane.

The mechanism of the cyclization appears essentially the same as that of azobenzene to benzo [*c*] cinnoline[3]. Azobenzene is known to undergo photocyclization only in presence of acids. In the case of 2-phenylazopyridine, there are evidences of cyclization to some extent even in neutral medium. This suggests some differences in certain aspects of the mechanism. This is being investigated in detail by studying the nature of the excited states involved.

Prof. S. S. Moosath is thanked for encouragement and the University of Calicut for facilities.

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1. Lewis, G. E., *J. Org. Chem.*, 1960, **25**, 2193.
2. Joshua, C. P. and Pillai, V. N. R., *Tetrahedron Letters*, 1972, p. 2493.
3. — and —, *Tetrahedron*, 1974, p. 3333.
4. Griffith, J., *Chem. Soc. Rev.*, 1972, p. 481.
5. Brown, E. V. and Granneman, G. K., *J. Am. Chem. Soc.*, 1975, **97**, 621.

NEW COLORIMETRIC ESTIMATION OF UREA WITH *p*-DIMETHYLAMINOBENZALDEHYDE

Methods available for the determination of urea are either time-consuming and cumbersome[1,2] or not applicable to samples containing high percentage of the diamide[3–7]. *para*-Dimethylaminobenzaldehyde has been found to give a bright greenish yellow colour with urea. The reaction is specific to urea and not affected by acetamide and biuret. No interference is observed with the common ions such as NH_4^+, Na^+, K^+, Ca^{++}, Cl^-, $SO_4^=$, NO_3^- and $H_2PO_4^-$ found in NK fertilizers. Hence the method finds application in the determination of urea in fertilizers. However, the final acid concentration in the colour-developed solution should be less than 0·13 N. Blank corrections are needed if HPO_4^{--} is present.

To an aliquot of urea solution containing 10–60 mg urea, 5 ml of 0·6% para-dimethylaminobenzaldehyde in 0·25–0·30 N HCl is added and the solution is made up to 25 ml with distilled water. The bright greenish colour developed is read at 420 nm. The colour is stable for quite few hours. The method is comparable to the conventional methods within ± 1·0%. The coefficient of variability of the method is 0·81% with repeatability of 2·2% of the urea present in the sample at the concentrations suggested elsewhere.

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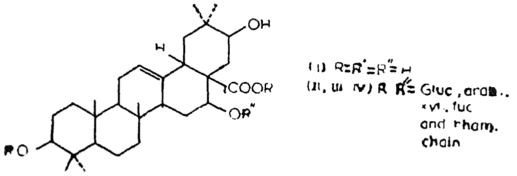
1. The Fertiliser (Control) Order, 1957 and the Essential Commodities Act, 1955, *Bulletin*, The Fertiliser Association of India, 1974.
2. *Specification for Urea, Technical and Pure*, Indian Standards Institution, IS: 1781–1961.
3. Karr, *J. Lab. Clin. Med.*, 1924, **9**, 329.
4. Koch, *Ibid.*, 1926, **11**, 776.
5. Allen, F. W. and Luck, J. M., *J. Biol. Chem.*, 1929, **82**, 693.
6. Looney, J. M., *Ibid.*, 1930, **88**, 189.
7. Overein, L., *Diss. Abstr.*, 1963, **24**, 1311.
8. Jain, J. M. and Sarkar, M. C., *Fertiliser News*, 1976, **21** (5), 20.

SONUSIDE—A NEW GLYCOSIDE FROM *ACACIA CONCINNA* D.C. BEANS WITHOUT SEEDS

During the search of natural glycosides of anti-tumour, anti-fertility, anti-cancer, and anti-cough and anti-rheumatism properties, it was found[1] that triterpenic as well as steroidal saponins with more than one sugar chain are the real glycosides found in nature which undergo partial hydrolysis or decomposition by heat, bacteria, enzymes and mild acids to give saponins of one sugar chain only. *Acacia concinna* D.C. (family

Leguminoseae, subfamily Mimoseae) commonly known as "Shikakai" was taken up to prove this point further. This prickly scandon bushy plant has been used[2] extensively for washing hairs, poisoning fishes, tanning purposes and preparing chutney.

A review of the literature showed that its seeds contain a pentacyclic triterpenic acid, acacic acid[3] (I), saponin acacinin[4] and two saponin glycosides[5]: Acacinin A (II) (major) and acacinin B (III). During the course of our research, the pods are also reported[6] to possess three different glycosides called acacinin C, D (major) (IV) and E.



The ether extract of the powdered *Acacia concinna* D.C. beans without seeds was fractionated into acidic (0·02%) and neutral fractions (0·00%) with 5% NaOH. The acidic fraction was crystallised, m.p. 265–266°, identified as acacic acid (m.p. 260–265°, acetate, m.p. 235–236°; showed no depression on m.m.p. with authentic samples). The beans on exhaustive extraction with aqueous methanol at low temperature, subsequent treatment with chloroform, carbon tetracholoride and benzene and extraction with *n*-butanol yielded a dark brown mass. TLC of this mass with solvent systems A[7], B and C and chlorosulphonic acid: acetic acid[8] as developer (1:2) revealed the presence of five spots (*cf*. Table I), which were separated by column chromatography on silica gel. The main glycoside fraction IV tentatively named "Sonuside" is absolutely different from all the so far reported glycosides and forms the basis of our studies. Fraction V is a mixture of free sugars.

TABLE I

Fraction No.	Solvent System*		
	A	B	C
I	0·91	0·95	0·94
II	0·72	0·78	0·75
III	0·35	0·40	0·38
IV	0·10	0·16	0·13
V	0·02	0·03	0·026

*A = Chloroform: Methanol: Water (65:35:10); B = (65:40:10).
C = Butanol: Acetic acid: Water (6:1:3).

Sonuside on crystallisation with aqueous methanol gave a very light brown fine powder, m.p. 193°C (dec.) $(\alpha)_D$ --13·4°; acetate, m.p. 175–176°C, $(\alpha)_D$ + 4·7°. Sonuside on partial hydrolysis yielded acacinin E (R_f. 0·18 in solvent system A)—a saponin of acacic acid with glucose, xylose, arabinose, rhamnose and fucose and an unidentified sugar as evidenced by paper chromatography, m.p. 200–201° (dec.), acetate, m.p. 135–136°. Sonuside on complete hydrolysis in dioxane-water with 3N HCl gave a residual mass, which was crystallised with methanol, m.p. 264–265° and identified as acacic acid. The filtrate on neutralisation over IRA–400 and concentration under vacuum on paper chromatography in ethyl acetate: pyridine: water (3·6 : 1 : 1·15) showed the presence of six sugars well comparable with authentic samples of glucose, arabinose, fucose, xylose and rhamnose and a faint spot of an unidentified sugar. IR spectra of Sonuside did not show any γ-lactone band at 1770 cm^{-1} whereas its acetate did show sharp absorption at 1765 cm^{-1}. The easy lactonisation in Sonuside during acetylation in cold, exactly like that in acacic acid, clearly shows that $C_{21}-OH$ and $C_{28}-COOH$ groups are free in Sonuside and no sugars are attached at these positions.

Sonuside on enzymatic hydrolysis with β-glucosidase yielded a colourless powder, identified as acacinin E, m.p. 200–201° (dec.). Thus Sonuside is a higher glycoside of acacinin E with β-D-glucose, which is linked at the end of the sugar chain at C_3 and/or at C_{16}. Hence acacinin E and so also acacinin D, reported earlier[6], are the artefacts and Sonuside is the real natural glycoside of *Acacia concinna* D.C. beans without seeds. The structure elucidation of Sonuside is in progress.

Thanks are due to CSIR (New Delhi) for the award of a Junior Research Fellowship to one of the authors (S. Walia), to The Head, Chemistry Department, for providing the facilities and Dr. S. S. Sandhu, Amritsar for I.R. spectra.

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1. Tschesche, R., Seidel, L., Sharma, S. C. and Wulff, G., *Chem. Ber.*, 1972, **105**, 3397.
2. *Wealth of India* (Raw Materials, C.S.I.R.), 1948, **1**, 13.
3. Varshney, I. P., Shamsuddin, K. M., and Beyler, R. E., *Tet. Lett.*, 1965, 1187; Varshney, I. P. and Shamsuddin, K. M., *Bull Chem. Soc. Japan*, 1970, **43**, 3830; Varshney, I. P. *et al.*, *Plianta Med.*, 1972, **22**, 447 and *Planta Med.* (in press).

4. Varshney, I. P.. and Sharma, S. C., *Indian J. Appl. Chem.*, 1969, **32**, 69.
5. — (Mrs.) Handa, G., Raj Pal and Srivastava, H. C., *Ind. J. Chem.*, 1976, **14 B**, 228.
6. —, Raj Pal and Srivastava, H. C., *J. Indian Chem. Soc.*, 1976, 153.
7. Kawasaki, T. and Miyahara, K., *Chem. Pharmac. Bull. (Tokyo)*, 1963, **11**, 1546.
8. Tschesche, R., Freytag, W. and Snatzke, G., *Chem. Ber.*, 1959, **92**, 3053.

SWARMING OF THE TINTINNIDS (PROTOZOA: CILIATEA) IN THE VELLAR ESTUARY

THE plankton collections were made intensively during August, 1976. The occurrence of 7 species of tintinnids, one of which formed a swarm, was noticed. On one occasion (18th September, 1976) twelve species of tintinnids occurred simultaneously in the plankton collections from the Porto Novo backwater, but no swarming was noticed; and in the previous report also no swarming was mentioned[1].

On 7th August, 1976, *Favella philippinensis* amounted to 1,28,58,000 organisms/m^3 out of the total tintinnid population of 1,33,26,000 Org./m^3. The total number of organisms in the plankton was 1,63,42,000 Org./m^3 made up of a phytoplankton component of 8,88,000 cells m^3. *F. philippinrnsis* constituted 96·5% of the total tintinnid population and 78·7% of the total plankton population. Surface water temperature and salinity values were 28·0° C and 30·60‰ at the time of swarming. *F. philippinensis* was common in the estuary. The other tintinnid species recorded along with *F. philippinensis* were *F. brevis*, *Coxliella ampla*, *Tintinnopsis tubulosa*, *T. tocantinensis*, *T. uruguayensis*, *Eutintinnus tenuis*, and *Metacylis jorgensenii*.

The associated species found with the tintinnids were the phytoplankton *Thalassiothrix frauenfeldii*, *Biddulphia sinensis*, *Dytilum brightwelli*, *Cheatoceros curvisetus*, *C. diversus*, *Rhizosolenia alata Coscinodiscus jonesianus* and *Acrocalanus* sp. and veligers. The coeval appearance of a large number of veligers with the tintinnids may be attributed to their food web links as the veligers were found to feed on the tintinnids.

It has been reported from the experimental studies that tintinnids showed higher rates of metabolism and reproduction[2]. A tintinnid concentration of 4,800 to 39,300 Org./m^3 has been reported from the Arabian Sea[3]. Swarming of tintinnids presently found could be due to abundant food supply, particularly diatoms, upon which they feed.

The minimum dimensions of the lorica of *F. philippinensis* presently observed were: total length 154 µm; horn length 14 µm; inner oral diameter 129 µm; the maximum dimensions presently observed were: total length 228 µm; horn length 45 µm; inner oral diameter 133 µm. On other occasions the lorical dimensions observed were (around) total length 250 µm; horn length 40 µm; inner oral diameter 110 µm.

It is quite likely that swarms of some more species of tintinnids may also occur.

We thank Prof. R. Natarajan, Director, Centre of Advanced Study in Marine Biology, for his keen interest and encouragement. We are grateful to Annamalai University and the University Grants Comission for facilities provided and for the financial support.

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1. Krishnamurthy, K. and Santhanam, R., *Indian J. mar. Sci.*, 1975, **4**, 181.
2. Gold, K., *Mar. Biol.*, 1971, **8**, 105.
3. Zeitzschel, B., *Meteor Forsch. Ergebn. Reihe, D.*, 1969, **4**, 47.

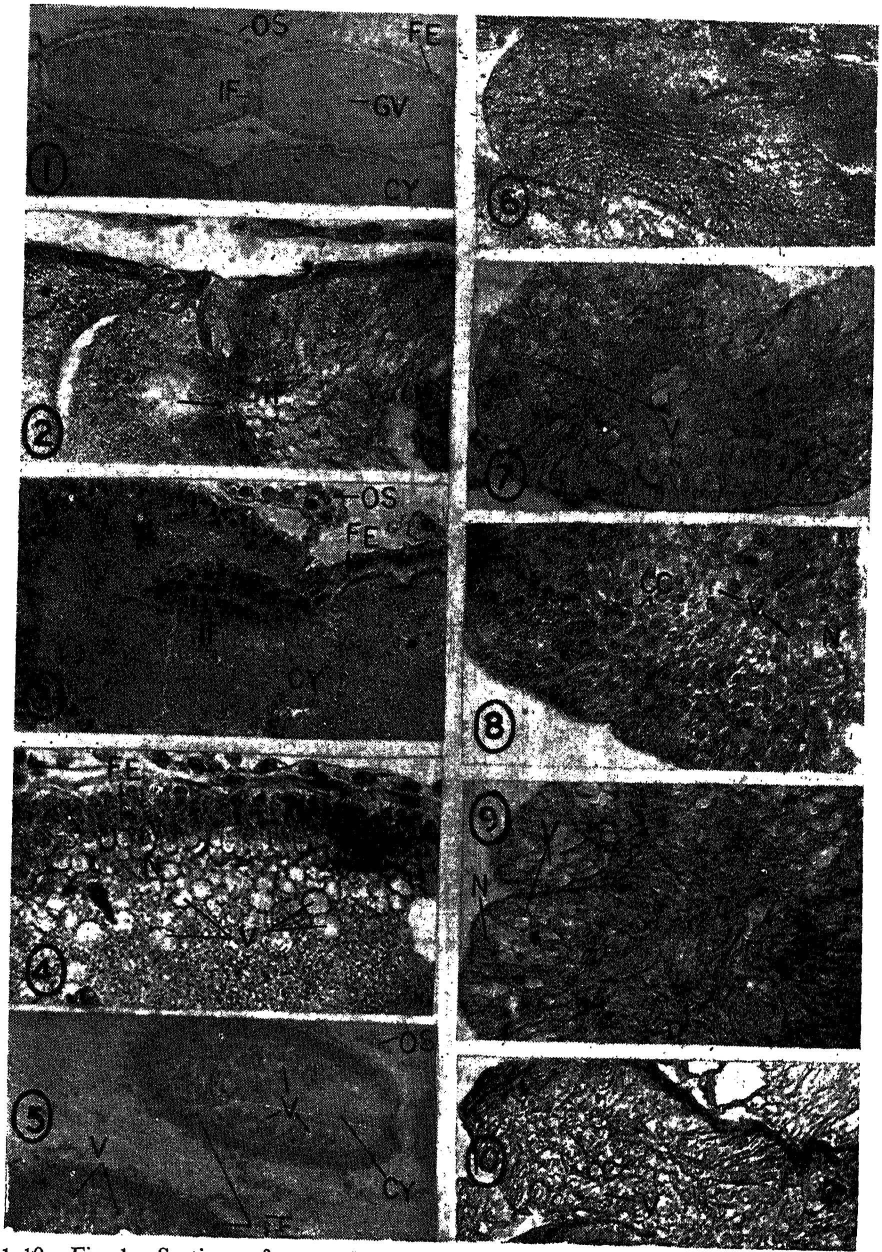
ON A POSSIBLE MODE OF ACTION OF HEMPA ON OVARIAN TISSUE *PERIPLANETA AMERICANA* L.

DURING the course of investigations on effects of various chemosterilants on neuroendocrine and reproductive system of *Periplaneta americana*, it was observed that hempa also damages the ovarian structure. The effect of hempa on corpora allata has already been reported by Bhargava and Mathur[1]. Tandon and Bhargava[2] opined that apholate perhaps damages the oocytes *via* corpora allata. The present paper deals with the effect of hempa on ovarian structure of *Periplaneta americana*. An attempt has also been made to correlate the damages of corpora allata and ovarian tissue in support of the hypothesis of Tandon and Bhargava[2].

The ovaries of the cockroaches whose corpora allata were used in the previous paper[1] were fixed in Bouin's fluid and sections were cut at 8 µ. Sections were stained with hematoxylin and eosin. Sections of ovaries of treated cockroaches were compared with similar preparations from untreated ones (Fig. 1)

The effects become apparent only after 48 h when a few vacuoles appear in peripheral cytoplasm of the oocytes. The vacuoles increase in number and size with lapse of time. After 96 h treatment germinal vesicle contents become granular. The interfollicular tissue starts thinning out. Later, it breaks (Fig. 3) to make the cytoplasm of adjacent immature oocytes continuous (Fig. 2).

Hempa damages mature oocytes more than the immature ones. The inner and outer cell boundaries of follicular epithelium cells of mature oocytes become thick and wavy (Fig. 5) while the lateral boundaries become more or less indistinct. Later, their cytoplasm is reduced, nuclei become prominent and



FIGS. 1–10. Fig. 1. Section of normal ovary, 10 × 10. Fig. 2. 72 h treated ovary showing the process of continuity of cytoplasm within adjacent oocytes, 10 × 40. Fig. 3. 120 h treated ovary showing the breaking of interfollicular tissue, 10 × 40. Fig. 4. 144 h treated mature oocyte showing vacuoles in the cytoplasm and karyorrhexis in the nuclei of follicular epithelium, 10 × 40. Fig. 5. 264 h treated mature oocyte showing vacuoles in the cytoplasm, 10 × 10. Fig. 6. Normal corpus allatum, 10 × 40. Fig. 7. 48 h treated corpus allatum showing beginning of the formation of vacuoles and clumping of the chromatin 10 × 40. Fig. 8. 72 h treated corpus allatum showing presence of vacuoles and clumped chromatin material in the nucleus, 10 × 40. Fig. 9. 144 h treated corpus allatum showing increased number of vacuoles and reduced amount of cytoplasm, 10 × 40. Fig. 10. 240 h treated corpus allatum showing vacuolated appearance and degeneration of the tissue, 10 × 40.

(CC, clumped chromatin; CY, cytoplasm; FE, follicular epithelium; GV, germinal vesicle; IF, interfollicular tissue; N, nuclei; OS, External ovariole sheath;

dumbbell-shaped (Fig. 4), A few nuclei show karyorrhexis. The external ovariole sheath breaks at many places. Granular fibrous degeneration could not be observed even after 23 days of treatment.

The experiment clearly shows that hempa's action resembles that of apholate, metepa and thiotepa as described by Tandon and Bhargava[2] in producing vacuolar degeneration and breaking the interfollicular tissue after thinning. The effect, however, is very slow and does not affect all the oocytes and ovarioles. It differs from all the three chemicals, in not producing granular and fibrous degeneration, possibly due to its different chemical nature. The experiment shows that hempa damages the nuclei more than cytoplasm. It is also interesting to note that only mature oocytes are damaged to a lagrer extent. Therefore, the possibility of overcoming the effect of chemosterilant cannot be ruled out. LaBrecque *et al*.[3] also mentioned the recovery of fertility after hempa treatment in female houseflies. Morgan[4] also expressed his doubt that the affected cells may be able to overcome the effect.

According to Lüscher and Engelmann[5] and Scharrer and von Harnack[6], a decrease in the amount of cytoplasm, presence of vacuoles and densely packed nuclei with clumped chromatin mass are indications of inactivity and degeneration of corpora allata. Exactly the same picture was obtained by Bhargava and Mathur[1] after hempa treatment. These authors reported that corpora allata show early signs of degeneration near about 48 h after the treatment when vacuoles appear in the peripheral cytoplasm (Fig. 7) and nuclei tend to move towards the centre. Almost at the same time the oocytes also show early signs of degeneration. Prolonged treatment increases the number of vacuoles (Figs. 8, 9 and 10) and the nuclei undergo pycnosis in corpora allata. Simultaneously, the ovarian tissue shows increased degeneration. After 240 h the size of corpora allata is reduced (Fig. 10) and the mature oocytes show a complete picture of degeneration. Allatectomy has similar effects in many insects. It causes oosorption in *Schistocerca*[7] and stops vitellogenesis in insects of various orders[8]. According to Girardie[9] egg maturation in *Periplaneta americana* also is controlled by corpora allata. A conclusion may, therefore, be drawn from the above experiment that the damage of the ovarian structure in *Periplaneta americana* is because of the inactivity of corpora allata which in turn is induced by hempa.

Thanks are due to United States Agriculture Department, U.S.A., for gift of hempa.

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1. Bhargava, S. and Mathur, R., *Chemosterilant News*, 1975, **3**(1), 13.
2. Tandon, G. N. and Bhargava, S., *Curr. Sci.*, 1977, **46**(5), 133.
3. LaBrecque, G. C., Morgan, P. B., Meifert, D. W and Fye, R. L., *J. Med. Ent.*, 1966, **3**(1), 40.
4. Morgan, P. B., *Ann. Entomol. Soc. Amer.*, 1967, **60**(4), 812.
5. Lüscher, M. and Engelmann, F., *Rev. Suisse Zool.*, 1955, **62**, 649.
6. Scharrer, B. and von Harnack, *Biol. Bull.*, 1958, **115**, 508.
7. Strong, L., *J. Insect Physiol.*, 1965, **11**, 135 and 271.
8. Engelmann, F., *The Physiology of Insect Reproduction*, Pergamon Press, Oxford, 1970.
9. Girardie, A., *J. Insect Physiol.*, 1962, **8**, 199.

SOME EXPERIMENTAL EVIDENCE ON THE VIABILITY OF *ASCARIS LUMBRICOIDES* OVA

Introduction

THE prevention of soil-transmitted helminthic infections is mainly a problem of night-soil disposal, which is a major health hazard in a country like India. Recently, sanitarians have evolved a special type of latrine, 'leach-pit bore-hole latrine', in order to produce acceptable manure by composting the night-soil. In view of the health hazard due to the presence of parasitic cysts and ova, people are reluctant to use the digested sludge as manure. It has been shown that even dried, and digested sludge from some sewage plants contains viable ova of *Ascaris*. Keller[5] (1951), Gotaas[4] (1956) and Malviya[7] (1964) reported that, of all the parasitic cysts and ova encountered in sewage sludges, the ova of *Ascaris* were the most resistant to composting. Such resistance is mainly attributed to the egg shell which consists of five layers, namely, an outer proteinous epithelial membrane, three layers of chitinous material and an inner fibrillar lipoidal membrane. Hence, in the present investigation, *Ascaris* ova have been used as the yardstick to assess the survival of parasitic organisms present in the sewage sludges. Experiments were carried out to determine the viability of *Ascaris* ova inoculated to digested sludge samples free from parasitic cysts and ova, using laboratory models, simulating the field conditions as in the leach-pit bore-hole latrines.

Although much work has been done on the influence of various factors, on the development of *Ascaris* ova, yet the findings of various workers regarding the viability of *Ascaris* ova in sewage sludges are at variance. Hence, an attempt has been made to assess, experimentally, the survival of *Ascaris* ova in sewage sludges using laboratory models.

Material and Methods

The viability of *Ascaris* ova in sewage sludges has been determined experimentally, by inoculating sludge samples with *Ascaris* ova and examining microscopically, as was done by Keller and Hide[6] (1951). Fertilised *Ascaris* ova isolated from 15 adult worms were inoculated to two sets of digested sludge samples free from any intestinal parasitic cysts or ova. The samples were transferred to pyrex glass tubes (24″ × 1·5″ dia). The tubes were wrapped with a black paper to avoid the probable effect of sunlight and mounted vertical as shown in Fig. 1. Precautions were taken to keep the sludge moist and to maintain the field conditions.

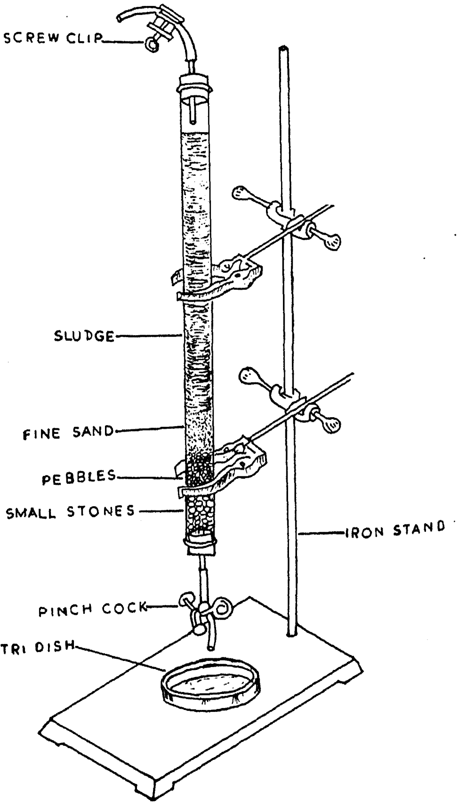

FIG. 1. Laboratory model used in the experiments.

Following the method of Malviya[7] (1964), viability of *Ascaris* ova was determined by isolating and culturing the ova at 37° C in a medium consisting of 5% formalin in physiological saline solution. Culturing and microscopic examinations were repeated at an interval of every two weeks. To determine the viability, the criterion used by Keller[5] (1951) was followed.

Results and Discussion

There has long been uncertainty as to the conditions which induce hatching of *Ascaris* ova. Several workers have investigated the survival or development of *Ascaris* ova in relation to different environmental factors such as temperature, season, etc., (Obha[9], 1923; Ogata[10], 1925; Brown[1], 1927; Faust[2], 1939; Gartner and Muting[3], 1951; Keller and Hide[6], 1951; Gotaas,[4] 1956; Susikaran[12], 1963; Malviya[7], 1964; Petrokova[11], 1967). During the present investigation, it has been observed that fertilised ova of *Ascaris*, under natural conditions, developed into active embryos within three weeks. Yoshida (1918) found that in favourable environment, embryos appear within the egg shell after a fortnight. Similarly, Manalang[8] (1927) observed that under experimental conditions, *Ascaris* ova developed into motile larva from 9 to 15 days at room temperature. Gartner and Muting[3] (1951) reported that *Ascaris* ova did not survive longer than 1½ years in land irrigated with sewage. Faust[2] (1939) reported the longest survival period of 5 to 6 years for the ova found in contaminated soil.

In the present study, the results of microscopic examinations proved that the viability of *Ascaris* ova is 50% after 1 year; 25% after 1½ years; 12% after 2 years and practically no survival after 3 years in the digested sludge sample. These findings are in conformity with the observations of Petrokova[11] (1967).

Although Susikaran[12] (1963) found viable *Ascaris* ova up to 14 months in leach-pit bore-hole latrine containing digested sludge, the results of the present investigation prove that, it is not safe to utilise the sewage sludge as manure, before a minimum period of three years after closing the pit. In view of the above findings, it is suggested, that appropriate regulations are enacted banning the use of wet sewage sludge as manure without allowing for a minimum period of three years after closing the pit.

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1. Brown, H. W., *J. Parasit*, 1927, **14**, 1.
2. Faust, E. C., *Human Helminthology*, 2nd Edition, Lea and Febiger, London, 1939, pp. 780.
3. Gartner, H. and Muting, L., *Ztschr. f. Hyg. u Infecktionskr*, 1951, **132**, 244.
4. Gotaas, *H. B.*, *Composting—Sanitary Disposal and Reclamation of Organic Wastes.* WHO Monograph Series, 1956, No. 31, pp. 205.
5. Keller, P., *J. Proc. Inst. Sew. Purif.*, 1951, Part I, p. 100.
6. —, and Hide, C. G., *South African, Med. J.*, 1951, **25**, 338.
7. Malviya, H. M., *Envir. Hlth.*, 1964, 6, 63.
8. Manalang, C., *Philippine. J. Sci.*, 1927, **33**, 249.
9. Obha, T., *Jap. Jour. Zoo.*, 1923, **1**, 120.
10. Ogata, S., *Ann. Trop. Med. Parasit.*, 1925, **19**, 301.
11. Petrokova, I., *Cslk. Hyg.*, 1967, **12**, 612.
12. Susikaran, M., *Envir. Hlth.*, 1963, **5**, 114.

IN VITRO POLLINIAL GERMINATION IN *CALOTROPIS*: POLARITY OF TUBE GROWTH AND ACTION OF GROWTH SUBSTANCES

THE occurrence of bag-like pollinia enclosing pollen grains in some members of Asclepiadaceae offers a new dimension to *in vitro* studies on pollen germination and tube elongation. The pollinial wall is made up of sporopollenin[1–3] except for a specific region (referred as a ' furrow ') through which the pollen tubes emerge during germination. This regional specificity of germination implies that tubes originating from all points inside the pollinium grow towards the furrow. It has been suggested[4] that the unidirectional growth of pollen tubes is due to the orientation of the germ pores of individual pollen grains towards the furrow or the germinating region. Alternatively, the polarity of tube growth may be due to the presence of chemotropic factors in and/or around the furrow. These assumptions are tested in the present study. Another aspect investigated here is the possibility of the pollinial wall altering or modifying the action of plant growth substances on the pollen grains it envelops.

The pollinia of *Calotropis gigantea* were selected for this investigation because of their large size and easy availability. Batches of 10 pairs of pollinia dissected out from fresh flowers of *Calotropis* were grown in distilled water, in the standard medium of Brewbaker and Kwack[5] and in standard media containing different concentrations (5, 10, 25, 50 and 100 ppm) of IAA, GA_3, NAA, 2, 4-D and kinetin. All experiments were conducted at 28° ± 2° C. Emergence of the first (first few) pollen tubes outside the pollinium is taken as the initiation of pollinial germination. In each trial, individual pollinia showed little variation in the time taken for germination. However, tube length attained after 24 hr showed some variability and the values compared were the mean of 60 measurements on the longest pollen tubes from each culture. The germinating region of the pollinia was cut out under the microscope and this portion as well as the rest of the pollinium were extracted separately. The usual paper chromatographic techniques were employed for the analysis of the extracts.

The pollinium germinates even in distilled water. Within 15 min of its contact with water, a slit-like structure begins to develop at the germinating region through which amorphous exudates ooze out and collect along the opening. In the exudates, pollen protoplasts with or without tubes are also seen. The germination time of pollinia in water is shorter than that needed in standard culture solutions but the tube growth in the former is slow and virtually stops after 2–3 hr. The maximum tube length measured is 180 μm in 24 hr or only about 5% of the length attained in standard culture solutions.

The pollen grains are non-aperturate. When imbided with water, the thin exine breaks irregularly and the intine protrudes out as the pollen tube. Frequently, the opening of the exine is wide enough for the entire pollen protoplast to move out. Exineless protoplasts escape from the pollinia when they are cultured both in water and in culture solutions; more of them, as a rule, occur in the former.

Pollen germination and tube initiation in *Calotropis* pollinia appear to be a direct result of water uptake through the furrow. Pollinia with their germinating region covered with water-proof materials like Fevicol produce no pollen tubes either in water or in culture solutions even after 24 hr. However, a slit cut out in the closed pollinial wall will lead to the emergence of pollen tubes through the new opening. Pollinia with the usual germinating region *plus* a slit made on the opposite side were also cultured. Here, tubes emerge from the openings on both sides with equal ease and grow with comparable rates of tube elongation. Further, chromatographic analysis of the germinating region and the rest of the pollinia shows no significant differences in chemical composition. These results suggest that the tubes would grow through any opening in the pollinial wall offering accessibility to water and rule out the possibility of : (*a*) any special arrrangement of pollen grains with reference to the germinating region and (*b*) the presence of chemotropic factors in the furrow guiding pollen tubes towards it.

None of the growth substances tested here promotes pollinial germnination (Table I). However, except for

TABLE I

Effect of various growth substances on pollinial germination and pollen tube elongation in Calotropis gigantea

Concentration ppm	Time required for initiation of pollinial germination (min)				
	IAA	GA_3	NAA	2, 4–D	Kinetin
0	30	30	30	30	30
5	30	90	60	60	60
10	30	90	60	60	60
25	30	90	60	30	30
50	30	90	60	60	30
100	30	90	60	30	30
	Pollen tube length attained in 24 hr (μm)				
0	3509	3831	4313	3492	3492
5	..	3904	3289	5365	7792
10	4190	7100	1175	3958	1663
25	6230	3517	615	3300	1474
50	3257	4654	89	2974	1734
100	2735	1663	85	823	1550

NAA, all of them at low concentrations enhance the rate of tube growth. The extent of their effect on tube growth is not identical, but as a rule, higher concentrations induce inhibition of growth.

Addicot[6] pointed out that pollen germination and tube elongation are distinct physiological processes depending on different factors. Later reports[7,8] on the effect of plant hormones on the pollen growth of loquat and tomato support this contention. The present findings also recognise two phases in pollen growth. As seen in Table I, most growth substances at low concentrations prolong the time needed for pollen germination but the same concentrations enhance tube elongation up to or over 200%. Thus, although the pollen grains of *Calotropis* are enveloped by the pollinial wall, they too exhibit characteristically different responses to growth substances during germination and tube elongation phases of early pollen growth.

Thanks are due to Prof. C. A. Ninan for facilities and to the UGC for a fellowship to one of us (P. S.).

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1. Jalaja, S. and Namboodiri, A. N., *Ind. Sc. Cong. Proc.*, Part III, 1975, 72.
2. — and —, *Experientia*, 1975, **31**, 915.
3. Vijayaraghavan, M. R. and Shukla, A. K., *Ann. Bot.*, 1976, 40, 417.
4. Galil, J. and Zeroni, M., *Bot. Gaz.*, 1969, **130**, 1.
5. Brewbaker, J. L. and Kwack, B. H., *Amer. J. Bot.*, 1963, 50, 859.
6. Addicot, F. T., *Plant Physiol.*, 1943, 18, 270.
7. Dikshit, N. N., *Curr. Sci.*, 1956, **25**, 27.
8. McLeod, K. L., *Ann. Bot.*, 1975, **39**, 59.

EVALUATION OF ROOTSTOCKS OF POME AND STONE FRUITS AND RELATED WILD SPECIES FOR RESISTANCE TO CROWN GALL

CROWN GALL, incited by *Agrobacterium tumefaciens* (Smith and Townsend) Conn, is an important disease of temperate fruit crops. In India, the writer has detected the prevalence of the disease on stone fruit trees in Himachal Pradesh[1] and Kumaon Hills of Uttar Pradesh. As the disease mostly attacks the underground and ground level parts of the plants, use of resistant rootstocks for commercial varieties of temperate fruits is deemed to be a suitable and effective control measure for the disease. A search for crown gall resistant rootstocks for pome and stone fruits was, therefore, considered an important and urgent manoeuvre to combat the disease in north-western hills of India. In order to identify sources of resistance, 24 stocks of pome and stone fruits and their related wild species were evaluated for their performance against the disease. The results of the evaluation are presented in this article.

Stocks of Malling Merton Series and species of the genera *Cotoneaster*, *Malus*, *Prunus* and *Pyrus* were included in the present investigation. A virulent culture of *A. tumefaciens*, isolated from galls on plum trees, was used as inoculum. At least 3 plants of each type were employed for evaluating the host reaction to the disease. Pot-grown, 2–3 year old plants were inoculated in the following manner: Crescentic annular wounds were first created on the stem at two sites—one at the soil level and another at 10–15 cm above soil—by knifing out a rectangular flap of bark and cortex of approximately 1 × 2 cm size. The wounded sites were then wrapped with absorbent cotton swabs soaked in aqueous suspension of 48 hr old culture of the bacterium (10^8 cells per ml). To avoid loss of moisture from the cotton swabs, the soil level and aerial inoculated sites were covered with soil and butter paper, respectively. Identically wounded plants but wrapped with water soaked cotton swabs served as checks. The inoculated and check plants were maintained in glasshouse at 17° to 28° C (24–26° C for about 18 hr a day). The inoculated sites were provided sufficient humidity for the development of the disease by keeping the cotton swabs moist. After 15 days, the cotton swabs were removed and regular observations were initiated for the development of the symptoms. The plants which failed to produce symtoms within 90 days of inoculation were reinoculated. Observations were continued for a period of 8 months.

On the basis of size and nature of galls produced at the inoculated sites, the plants were evaluated as susceptible, moderately resistant or resistant. In susceptible plants, the galls were characteristically active and attained conspicuously large size (diameter more than 1 cm) within 90 days. The galls continued to show peripheral meristematic activity for the whole period of observation. The galls produced on moderately resistant plants were comparatively smaller in size (less than 1 cm in diameter after 8 months) and did not show any peripheral meristematic activity. The growth of the galls was extremely slow on these plants. Failure of the plants to support any tumourous activity at the inoculated sites was designated as resistant reaction to the disease.

Of the 24 rootstocks tested, susceptible reaction was shown by the plants of *Cotoneaster microphylla* Wall., *Malus pumila* Mill., *M. sikkimensis* (Hook. f.) Kochne, Malling Merton 101, 102, 105, 106, 109, 110, 115, *Prunus amygdalus* Batsch, *P. armeniaca* L., *P. avium* L., *P. cerasus* L., *P. cerasoides* D. Don (syn. *P. puddum* Roxb.), *P. cornuta* Stend. (syn. *P. padus* L.), *P. domestica* L., *P. persica* (L.) Batsch., *P. salicina* Lindl., *Pyrus communis* L. and *P. pashia* Buch-Ham. Moderate degree of resistance was detected in the stocks of *Cotoneaster acuminata* Lindl., *C. bacillaris* Wall. and *Malus baccata* (L.) Borkh. None of the stocks could be rated as resistant to the disease.

The results of inoculations reveal that the plum isolate of *A. tumefaciens* is pathogenic to many plant species, indicating thereby that the disease would be occurring on plants of pome and other stone fruits in Himachal Pradesh. It is a major concern that none of the rootstocks, tested in the present studies, could be evaluated as resistant to the disease. Plants of *C. acuminata* and *C. bacillaris*, identified as moderately resistant to crown gall, may be tried as rootstocks for pear. Availability of moderate degree of resistance in rootstocks of *M. baccata* may help to avoid a situation of crown gall becoming a potential threat to apple cultivation.

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1. Durgapal, J. C., *Indian Phytopath.*, 1971, **24** (2), 379.

A PEARL MILLET STRAIN WITH $2n = 12 + 4$ TELOCENTRIC CHROMOSOMES

IN the progeny of an autotriploid *Pennisetum typhoides* (Burm) S and H, in a population of 227 plants, 4 plants showed a deviant karyotype from the normal one. The normal chromosome number for this species is $2n = 14$, but these plants are with chromosome number $2n = 13 + 2$ fragments. These fragment chromosomes on further study proved to be telocentrics. At mitotic metaphase these two fragment chromosomes showed terminal centromeres and they corresponded in their lengths to the two arms of one of the odd chromosomes in the complement which was the longest chromosome of the complement with sub-median centromere. The two telos were slightly unequal in size. At meiosis these two telos paired with one of the normal chromosomes to form a heteromorphic trivalent. This trivalent was always a chain type with the two telos associated with the two arms of the normal chromosome. They were never observed to pair among themselves. At metaphase I, the orientation of the trivalent was non-random and resulted at Anaphase I segregation with the normal chromosome moving to one pole and the two telos to the other pole (62%).

When these plants were selfed, in the progeny plants with $2n = 12 + 4$ telos were obtained together with aneuploids and diploids (Table I).

TABLE I

Chromosome constitution of plants in the progeny of a plant with 13 + 2 *telos*

Chromosome constitution	14	13+2 telos	12+4 telos	14+1 telos	Total
Number of plants	12	51	14	1	78

The plants with 12 + 4 telos are of interest. These plants are obviously the result of the union of gametes with chromosome numbers $n = 6 + 2$ telos. In these plants two of the telos were longer than the other two telos. The four telos regularly formed two rod bivalents at Diakinesis and Metaphase I (Table II) (Figs. 1, 2 and 3).

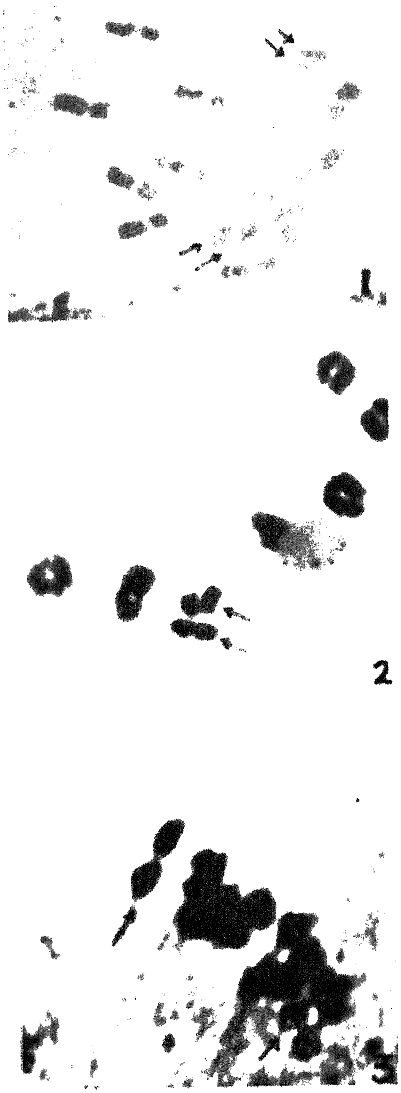
In 364 cells out of 396 cells observed at Diakinesis, the 4 telos regularly formed 2 rod bivalents and in 31 cells they formed 1 bivalent + 2 univalents. Only in 1 cell, the four telos remained as univalents. The mean chiasma frequency per cell at Diakinesis was 12·803 and the variance was 1·066.

TABLE II

Summary of chromosome associations in plants with 12+4 *telocentric chromosomes*

Stage of Meiosis	Normal chromosomes			Telocentrics		Total number of cells
	Bivalents		Univalents	Bivalents	Univalents	
	Ring	Rod				
Diakinesis	4·742	1·132	0·035	1·922	0·156	396
Metaphase I	3·896	1·821	0·434	0·963	0·433	106

At Anaphase I the distribution of chromosomes observed were 8–8 and 9–7 with frequencies 50 and 10 respectively in a total of 60 cells examined.



Figs. 1–3. *Pennisetum typhoides* (Burm) S & H. Fig. 1. Karyotype showing 2*n* = 12 + 4 telos (Arrows indicating the two pairs of telos). Fig. 2. Diakinesis showing 5 ring bivalents and 3 rod bivalents of which two are formed by telos. Fig. 3. Metaphase I showing 6 bivalents formed by standard chromosomes and two by telos.

The transmission frequencies of the telos from the male and the female side were determined by performing reciprocal crosses with normal diploids. The results are presented in Table III.

TABLE III

Chromosome constitution of the progeny plants obtained from the crosses of plants with chromosome number 2n = 12 + 4 telos

Cross	13 + 2 telos	13 + 3 telos	12 + 4 telos	Total
12 + 4 × 14	38	1		39
14 × 12 + 4	22			22
12 + 4 selfed			23	23

For four generations on selfing the plants with chromosome number 2*n* = 12 + 4 telos gave rise to progeny plants with 2*n* = 12 + 4 telocentric chromosomes only. That is this karyotype has become stable with telocentrics. These plants in their morphology were not different from the diploid sibs or from the plants with chromosome number 2*n* = 13 + 2 telos.

Over the question of stability of telocentric chromosomes which arose either spontaneously or in experiments, there was some controversy. Nawaschin[1] was the first to suggest that the chromosomes with strictly terminal centromeres could not survive in nature and for many years this remained the generally accepted view. It was argued by those who rejected the concept of telocentric condition, that all stable rod chromosomes were acrocentric and the evidence available at the time seemed to support this interpretation. Thus it was believed that minute heads which are often seen at the centric end of the rods clearly demonstrated the existence of short arms (Darlington[2], White[3]). It was also pointed out that when telocentrics either arose spontaneously (Upcott[4]) or following X-ray irradiation (Rhoades[5]), through the fracture of the interstitial centromere, their unstable behaviour usually led to their rapid elimination.

With the improvement of the techniques of chromosome analysis, there has been evidence that not all such chromosomes are acrocentric. With the available information on the structural organization of the centromere (Lima de Faria[6]), with a degree of certainty it has been shown that in many rod chromosomes, the so called 'small arms' represent fused centromeric chromomeres (Southern[7]). In addition there are now several recorded examples of derived telocentrics in plants which are stable (Darlington and La Cour[8]; Marks[9]; Steinitz[10]; Tanewaki[11] and Tsuchiya[12]). It was further suggested that telocentrics are rare not because their centromeres are inefficient but because they have a low chance of survival on account of genetic imbalance, it is likely that many of the cells die (Marks[9]).

In the present material, the presence of four telocentrics in place of two full chromosomes makes the chromosome complement full and their presence is not affecting either the exophenotype or the endophenotype. Their behaviour clearly shows the genetically well balanced structure of this strain. The meiosis is normal giving rise to genetically balanced gametes largely. Thus the telocentrics in this strain could be stable.

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---

1. Nawaschin, S. G., *Timirjasseffs Festschrift*, 1916, p. 185.
2. Darlington, C. D., *J. Genet.*, 1936, **33**, 465.
3. White, M. J. D., *Proc. Roy. Soc.*, 1935, **B 119**, 61.
4. Upcott, M. B., *Ibid.*, 1937, **B 124**, 336.
5. Rhoades, M. M.. *Genetics.*, 1936, **21**, 491.
6. Lima-de-Faria, A., *Hereditas* (Lund.), 1956, **42**, 85.
7. Southern, D. I., *Chromosoma (Berl.).* 1969, **26**, 140.
8. Darlington, C. D. and Lacour, L. F., *Heridity*, 1950, **4**, 217.
9. Marks, G. E., *Amer. Naturalist*, 1957, **91**, 223.
10. Strid, A., *Bot. Not (Lund.)*, 1968, **121**, 153.
11. Tsunewaki, K., *Can. J. Genet. Cytol.*, 1963, **5**, 462.
12. Tsuchiya, T., *Cereal Research Commu.*, 1973, **1** (3), 23.

A NEW SPECIES OF *MICROPERA* LEV. (COELOMYCETES) FROM MAHARASHTRA (INDIA)

A RARE but interesting pycnidial fungus belonging to the form-genus *Micropera* Lev. (Form O. sphaeropsidales) was recently collected at Poona growing on *Kickxia ramosissima* (Wall.) Janchen. (Fam. Scrophulariaceae) well known for its medicinal properties. On detailed study of its morphology and dimensions the fungus proved to be quite distinct from all other known species of the form-genus including *M. indica* Paw. and Kulk[1]. Hence, the same has been described here as a new species, with Latin diagnosis thus:

Micropera suttonii sp. nov. (Fig. 1)

Stromata atro-fusca, sub-erumpentia, dispersa, pycnidia dispersa, multi-loculata (2–3), crassiusculae ostiolata, immersa in plantis textibus, pezizoidea, magnitudine, 290–360 µm in diam. Pycnidiophora brevia, simplicia, hyalina vel sub-hyalina. Pycnidiospores cylindraceo-fusoidea, 3–5 septata, curvata vel flexa, hyalina vel olivacea, magnitudine, 58–86 × 3·3–4·2 µm.

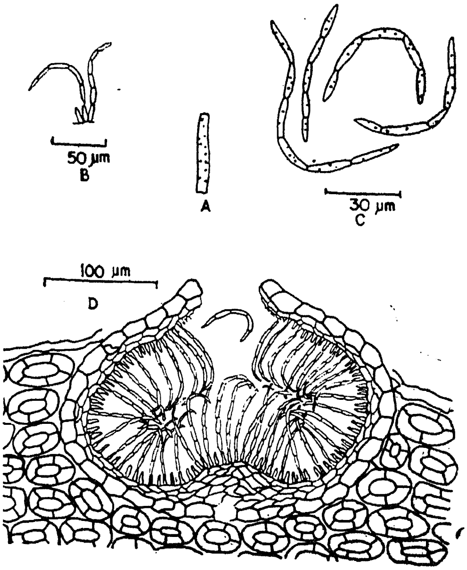

FIG. 1. *Micropera suttonii* sp. nov. A, Habit; B, Conidiophores; C, Pycnidiospores; D, V.S. of Pycnidium.

Matrix: In culmis viventibus *Kickxia ramosissima* (Wall.) Janchen. (Fam. Scrophulariaceae), ad Poona, India, 26-6-1976, legit B. R. Dayakar Yadav. No. AMH 3388 (Holotypus).

The specific epithet has been chosen in honour of Dr. B. C. Sutton, Mycologist, C.M.I., Kew, England, in recognition of his notable contributions to Coelomycetes.

Discussion

On critical study of morphological features of the 16 known species of *Micropera* Lev., the pycnidiospores of the present collection were found to be significantly bigger in size being 58–86 × 3·3–4·2 µm and hence the fungus is presented here as a new species. The fungus constitutes the 2nd report from Maharashtra.

The authors are grateful to Prof. M. N. Kamat, Head, Department of Mycology and Plant Pathology, for his keen interest and to the Director for the laboratory facilities.

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---

1. Pawar, I. S. and Kulkarni, U. K., *J. Bombay Nat. Hist. Soc.*, 69 (2). 457.

A NEW SPECIES OF *CONIOCHAETIDIUM* FROM INDIA

DURING studies of coprophilous fungi a large number of ascomycetes were isolated along with other fungi. From tiger excreta collected from zoo at Gwalior, an interesting fungus was isolated and identified as a species of *Coniochaetidium* Malloch and Cain. The genus *Coniochaetidium* was recently erected by Malloch and Cain[1] to accommodate two species, *i.e.*, *C. savoryi* (Booth) Malloch and Cain, and *C. ostreum* Malloch and Cain. They differentiated these species on the basis of shape and size of ascospores. The genus *Coniochaetidium* and *Coniochaeta* were kept together by Malloch and Cain[1] in a separate family Coniochaetaceae which was distinguished from Sordariacae by the presence of ascospores with elongated germ slits and from Xylariaceae in lacking stromatic tissue[2].

The species described in this paper differs from the other two species[1] in its characteristic 4-spored, glabose asci and lenticular ascospores. The ascus wall in the present isolate is somewhat hyaline and evanescent after the maturation of the ascospores. It differs from *C. savoryi* in having larger ascospores, strongly flattened on one side. It differs from *C. ostreum* in having ascospores which are nearly double its length and flattened on one side only. The fungus in question is, therefore, described here as a new species of *Coniochaetidium*—*C. coprophilum* sp. nov. (Fig. 1).

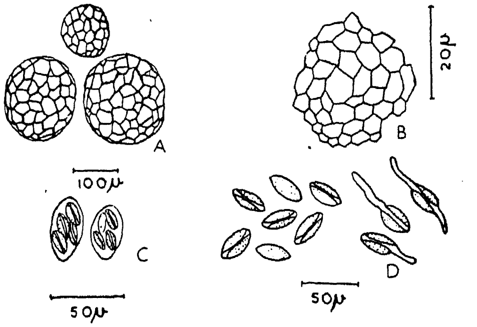

FIG. 1. *Coniochaetidium coprophilum*, IMI 179850. A. Cleistothecia; B. Part of peridium; C. Asci with ascospores; D. Ascospores showing germination.

Mycelium vegetabile floccosum, tenue, ramosum, septatum griseum. Ascocarpi sporas matures includentes post diem duodecimam evoluti. Cleistothecia sparsa vel laxe aggregata, hyphis pluribus griseolis flexuosis circumdata, globosa, atra, 65·5–59·8 µm diametro, peridermio obscure brunneo, e cellulis forma irregularibus in strato unico expansis composito. Asci globosi, leviter tunicatae 4-sporae, 32–56 × 7·4–14 µm. Ascosporae immaturae griseo-flavae, maturae in colorem succineum vergentes, lenticula ornatae, 19·95–28 × 13·3 µm. Status conidialis haud notatus.

Cultura typica posita in Ind. typ. cult. Coll. (1869) in I.A.R.I., ad New Delhi. Culturus typicus IMI 179850 est.

Vegetative mycelium greyish, floccose, hyphae thin, branched, septate. Ascocarp with mature ascospores developing after 12 days of incubation. Cleistothecia scattered or loosely clustered, covered with numerous greyish flexuous hyphae, globose, dark black in colour, 66·5–59·8 µm in diameter. Cleistothecia with dark brown peridium formed a single layer of cells which are irregular in shape. Asci globose, smooth-walled, 4-spored, 32–56 × 7·4–14 µm. Young ascospore greyish yellow in colour at maturity, turning amber in colour, lenticular, with an equatorial germ slit, 19·95–28 × 13·3 µm. No conidial state was observed when grown on PDA.

Fungus grows well on PDA. Colony diameter reaches upto 8 cm after 10 days of incubation at 28° C. The fungus is also grown on Czepek's-, malt- and yeast extract agars where the growth is not as luxuriant as on PDA. Germination of ascospores takes place by splitting through the germ slit resulting in one to two germ tubes.

A culture of the fungus has been deposited at Commonwealth Mycological Institute, Kew, England, under reference No. IMI 179850 and at I.A.R.I., New Delhi, India, No. 1869.

The authors are grateful to Dr. Hawksworth and Director, CMI, Kew, England, for their help in confirming the identity of this fungus. We acknowledge our sincere thanks to Prof. S. B. Saksena, F.N.A., Department of Botany, University of Saugar, Sagar, for providing laboratory facilities, to Prof. Roy. F. Cain, University on Toronto, Ontario, for supplying the necessary literature and to Dr. D. P. Rogers, University of Illinois, for Latin diagonosis.

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---

1. Malloch, D. and Cain, R. F., "New cleistothecial Sordariaceae and a new family, Coniochaetaceae," *Can. J. Bot.*, 1971, **49**, 869.
2. Martin, P., "Studies in the Xylariaceae. I. New and old concepts," *J.S. Afr. Bot.*, 1967, **33**, 205.

REVIEWS AND NOTICES OF BOOKS

Binary and Multiple Systems of Stars. By Alan H. Batten. (Pergamon Press, Oxford, England). Pp. i–xii + 278. Price $16.75.

The book is primarily devoted to binary systems with an emphasis on observational results. As intended by the author, a good knowledge of modern astrophysics is assumed as a prerequisite. However, matters specifically pertaining to binary systems are dealt with in detail, in the initial chapters. The author has clearly wished to avoid duplication of the readily accessible previous work; therefore in a majority of cases, theoretical results which could be readily supplemented from other sources are given without derivation.

The multiple star systems get only one chapter, but it is perhaps the first time that even so much space has been allotted to such systems. Furthermore, a small but constant undercurrent of the interrelations between binaries and multiples very nicely permeates most of the book.

There is one chapter each on the periods of binary systems, the stellar masses and radii in such systems, and the apsidal motions.

The theory of stellar atmospheres has an important bearing on the observations of binaries. This is discussed in the next chapter. The following two chapters respectively contain discussion of the observational evidence for circumstellar matter in binary systems and the models for them.

The last chapter is devoted to the evolution and origin of binary systems. The Algol paradox, and its by now well-known resolution in terms of mass-transfer is presented along with later developments.

Fairly extensive references are provided on all the topics. The discussions of observational results are accompanied by a critical assessment taking into account various sources of uncertainties which one must cope with.

In all, this book is a very welcome addition to the few existing monographs on binary systems.

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Elements of Group Theory for Physicists. 2nd Edition By A. W. Joshi, Institute of Advanced Studies, Meerut University. (Wiley Eastern Ltd., New Delhi) 1977. Pp. 324. Price Rs. 45·00.

Group theory as a subject has become an indispensible part of physics and chemistry. This book is an introductory text which explains the foundations of the subject and some of its physical applications.

The book is divided into eight chapters. The aspects of group theory are developed in the first four chapters: Abstract group theory, Hilbert spaces and operators, Representation theory of finite groups, and Continuous groups and their representations. In the next four chapters, the individual chapters that survey group theory's applications in various areas of physics and chemistry: Group theory in quantum mechanics I and II, Crystallographic and molecular symmetries, and Group theory in solid state physics. Several problems have been suggested towards the end of each chapter, which should prove useful. There are also three appendices: Elastic constants of crystals, Piezoelectricity and dielectric susceptibility, and Time-reversal symmetry and degeneracy.

All the chapters are well written and the author has given lucid explanations. The emphasis is placed on clear presentation of the essentials of the subject-matter rather than the mathematical rigor.

This book will be valuable to post-graduates and research workers in physics, especially chemical physics, solid state physics and physical chemistry, wishing to familiarize themselves in the field of group theory.

D. N. S.

6480/77. Published by S. R. S. Sastry, Manager, Current Science Association, Bangalore-560006
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CONFORMATIONAL ANALYSIS OF GLYCOPROTEINS

Part I. Conformation of the Protein Segment at the Site of Peptide-sugar Linkage

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ABSTRACT

The prediction of the secondary structure of about 55 Glycoproteins which contain Asn (CHO)–X–Ser/Thr, Asp (CHO)–X–Ser/Thr, Ser(CHO)–X–Y, and Thr(CHO)–X–Y was carried out by using the methods of Chou and Fasman. In most of the cases amino acid sequence at the site of sugar linkage assumes a β-turn. The present analysis also suggests that the nature of the amino acid residue X [Asn(CHO)–X–Ser/Thr, Asp(CHO)–X–Ser/Thr, Ser(CHO)–X–Y and Thr(CHO)–X–Y] plays a major role in deciding whether all or part of the amino acid residues in these sequences are involved in the β-turn. In Aspartic acid linked sugars, the triplet sequence Asp(CHO)–X–Ser/Thr is conserved [like in Asn(CHO)–X–Ser/Thr] and bend begins with Aspartic acid (though such examples are very few), thus involving the complete triplet sequence in the formation of the β-turn. In proteins which contain Ser(CHO)–X–Y and Thr(CHO)–X–Y sequences, the amino acid residue preceding the above sequences is generally either Pro or Gly. These results are in disagreement with the 'code sequence'. Thr/Ser (CHO)–X–Y–Pro– proposed for the linkage of sugar to Threonine or Serine from the earlier studies.

GLYCOPROTEINS belong to an important group of biopolymers, which occur in connective tissues, blood cells, serum, urine, saliva and other body fluids and serve various biological functions. They consist of essentially a protein backbone to which carbohydrates are linked covalently. They differ not only in the relative proportions of types of sugar but also in the number of side chains present. The frequency of occurrence of oligosaccharides along the peptide chain also varies markedly from one protein to another. Out of the twenty different amino acids, only L-asparagine, L-serine, L-threonine, L-aspartic acid are common protein constituents which form covalent linkages with carbohydrates. In glycoproteins where carbohydrate is linked to asparagine, a common sequence Asn(CHO)-X-Ser(Thr) has been observed. Such sequences are also found in proteins which lack the sugar. The importance of occurrence of Ser(Thr) in the third position has been explained by Marshall and Neuberger[1] who suggested a hydrogen bond between the carbonyl group of the side chain of asparagine and the hydroxyl group of hydroxy amino acid, to decrease the dissociation constant of the amide group, thereby facilitating the attachment of sugar to asparagine residue. However nothing is known about the possible conformation of the protein chain around the site of peptide-carbohydrate linkage and the nature of the amino acid residues. Such information may throw light on the stereochemical requirement at the site carbohydrate-protein linkages.

Method of Calculation: Recently Fasman and Chou[2–4], from the analysis of the 20 naturally occurring amino acids proposed a set of parameters P_α, P_β, P_c and p_t for prediction of protein conformation based on the frequency of occurrence of amino acids in the α-helical, β-sheet, coil and β-turn regions respectively from the known protein structures. These parameters have been used to assign the regions of α and β, coil and β-turn regions. The relative probability p_t of a tetrapeptide to form a β-turn was computed from the frequency of occurrence of each residue at the 1st, 2nd, 3rd and 4th positions[4]. The cut off value of β-turn[4] was chosen as $1\cdot6 \times 10^{-4}$. In cases, where the complete sequences are not known, as far as possible, at least three amino acid residues, on either side of an amino acid to which sugar is attached were considered. The amino acid residues that are involved in the β-turn are underlined by the solid line. The average parameters $\langle P_t \rangle$, $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ computed for the tetrapeptide involved in the β-turn, following the procedure outlined by Fasman and Chou are also shown in Tables I, II, III and IV. These average values are obtained by adding the individual P_α, P_β and P_t values of the residues in the tetrapeptide under consideration and dividing by four, the number of residues involved.

RESULTS AND DISCUSSION

The amino acid sequences around carbohydrate-peptide linkages of several glycoproteins are displayed in Tables I, II, III and IV. It is seen from Table I that mostly in glycopeptides with GlcNAc-Asn group, the sequence is either Asn-X-Thr or Asn-X-Ser. However very few exceptions have also been observed where the third residue is not a hydroxy amino acid. This indicates that exceptions to the suggestions of Marshall and Neuberger do occur (though rarely) where glycosylation of the asparagine takes place without the presence of hydroxy amino acid, serine or threonine in the third place of the sequence Asn-X-Thr(Ser). It

TABLE I

$\langle P_t\rangle$, $\langle P_\beta\rangle$, $\langle P_\alpha\rangle$ and P_t values of glycopeptides at the site of sugar linkage to asparagine residue

Glycoprotein (1)	Amino acid sequence at the site of sugar linkage (2)	$p_t \times 10^4$ (3)	$\langle P_t\rangle$ (4)	$\langle P_\beta\rangle$ (5)	$\langle P_\alpha\rangle$ (6)
Porcine Luteinising hormone-α-subunit	-Arg-Val-Glx-$\overset{*}{\text{Asn}}$-Ser-Thr-Gln[5]	2·20	1·27	0·95	0·88
Follicle stimulating hormone-β-subunit	-Leu-Thr-Ile-$\overset{*}{\text{Asn}}$-Thr-Thr-Trp[5]	3·78	1·21	1·06	0·88
β-lipotrophic hormone	-Asp-Arg-Ser-$\overset{*}{\text{Asn}}$-Ala-Thr-Leu[5]	2·98	1·34	0·77	0·82
Bovine Prothrombin	-Tyr-Arg-Gly-$\overset{*}{\text{Asn}}$-Val-Ser-Val[5]	2·57	1·28	0·91	0·67
	-Pro-Glu-Ile-$\overset{*}{\text{Asn}}$-Ser-Thr-Thr	2·20	1·32	0·94	0·79
	-Trp-Asp-Lys-$\overset{*}{\text{Asn}}$-Phe-Thr-Val	1·95	1·07	0·87	0·98
Lamprey Fibrinopeptide	-Ala-Thr-Asn-$\overset{*}{\text{Asn}}$-Ser-Asp-Pro[5]	3·18	1·43	0·81	0·77
		3·13	1·44	0·70	0·77
Deoxy Ribonuclease A, B, C	-Lys-Met-Ser-$\overset{*}{\text{Asn}}$-Ala-Thr-Leu[5]	1·67	1·12	1·00	1·04
Stem Bromelain	-Gln-Ser-Asn-$\overset{*}{\text{Asn}}$-Glu-Ser-Ser[5]	2·10	1·32	0·95	0·57
Transferrin	-Leu-Ile-His-$\overset{*}{\text{Asn}}$-Arg-Thr-Gly[5]	2·89	1·30	0·72	0·89
Haptoglobin β-chain (human)	-His-Gln-$\overset{*}{\text{Asn}}$-Asn-Ser-Thr-Ala[5]	3·66	1·21	0·81	0·97
		2·47	1·43	0·81	0·77
Bovine Thrombin B chain	-Phe-Lys-$\overset{*}{\text{Asn}}$-Pro-Thr-Val[5]	1·68	1·12	1·03	0·82
Porcine Pancreatic Lipases L_A and L_B	-Thr-$\overset{*}{\text{Asn}}$-Gly-Thr-Ile-[6]	2·34	1·34	0·97	0·73
Human and Porcine Ceruloplasmin	-Ala-Ile-Tyr-$\overset{*}{\text{Asn}}$-Asp-Thr-Thr[7]	2·25	1·28	0·99	0·79
Phosvitin	-Ser-$\overset{*}{\text{Asn}}$-Ser-Gly[17]	3·28	1·51	0·73	0·71
Phospholipase A[a] (honey bee)	-Gly-His-Gly-$\overset{*}{\text{Asn}}$-Lys-Ser-Ser[25]	5·13	1·21	0·73	0·89
		3·16	1·36	0·71	0·85

* denote the site of attachment of sugar residue to the amino acid.

[a] denote the site of sugar linkage is not certain.

[b] numbers on the top of the amino acid sequence denote the references.

[c] the amino acids that are involved in a β-turn are underlined.

is also interesting to note (Table IV) that in general the third amino acid residue is also a hydroxy amino acid, in aspartic acid linked carbohydrate moieties [Asp (CHO)-X-Ser/Thr]. On the other hand that in Ser (CHO)-X-Y, Thr(CHO)-X-Y, Y need not necessarily be a hydroxy amino acid (Tables II and III).

The p_t values of the amino acid sequences (Tables I–IV) at the site of sugar linkage are mostly higher than $1 \cdot 6 \times 10^{-4}$ and the average value of $\langle P_t\rangle$ is higher than $\langle P_\alpha\rangle$ and $\langle P_\beta\rangle$ suggesting that the peptide chain at the site of sugar linkage assumes a β-turn whether the sugar is linked to Asn, Thr, Ser or Asp. Similar results have

TABLE II

$\langle P_t\rangle$, $\langle P_\beta\rangle$, $\langle P_\alpha\rangle$ and p_t values of glycopeptides at the site of sugar linkage to serine residue

Glycoprotein	Amino acid sequence at the site of sugar linkage	$p_t \times 10^{-4}$	$\langle P_t\rangle$	$\langle P_\beta\rangle$	$\langle P_\alpha\rangle$
Human Chorionic Gonadotropin–β-subunit	$\text{–Ser–}\underline{\text{Ser–Ser–}\overset{*}{\text{Ser}}\text{–Lys}}\text{–Ala–Pro}^{5}$	3·16	1·34	0·73	0·86
	$\text{–Pro–}\underline{\text{Pro–Pro–}\overset{*}{\text{Ser}}\text{–Leu}}\text{–Pro–S}$	4·16	1·18	0·80	0·83
	$\text{–Pro–}\underline{\text{Ser–Pro–}\overset{*}{\text{Ser}}\text{–Arg}}\text{–Leu–Pro}$	5·83	1·31	0·74	0·74
	$\text{–Pro–Gly–}\underline{\text{Pro–}\overset{*}{\text{Ser}}\text{–Asp–Thr}}\text{–Pro}$	3·79	1·33	0·84	0·80
Hinge region of IgA$_1$ Myeloma Protein (Pat, Ose)	$\text{–Pro–}\underline{\text{Thr–Pro–}\overset{*}{\text{Ser}}\text{–Pro}}\text{–Ser–Thr}^{30}$	3·14	1·33	0·79	0·70
Carbohydrate units of IgA$_1$ Immuno-globulin	$\text{–Pro–Thr–Pro–}\overset{*}{\text{Ser}}\text{–Pro–Ser–Thr–}$	3·14 6·45	1·33 1·36	0·79 0·82	0·70 0·75
	$\text{–Pro–}\underline{\text{Thr–Pro–}\overset{*}{\text{Ser}}\text{–Pro}}^{31}$	3·14	1·33	0·79	0·70
Fetuin Glycopeptides	$\text{–Gly–Pro–}\overset{*}{\text{Ser}}\text{–Pro–Thr–Ala}^{21}$	3·59 2·12	1·43 1·18	0·69 0·88	0·63 0·91
	$\text{–Gly–}\underline{\overset{*}{\text{Ser}}\text{–Thr–Pro–Gly}}$	1·22	1·38	0·84	0·68
Disease Protein ZUC	$\text{–}\underline{\text{Pro–Gly–Gly–}\overset{*}{\text{Ser}}}\text{–Ser–Glu–Pro}^{32}$	4·21	1·48	0·74	0·61
Bovine submaxillary Mucin	$\text{–}\underline{\text{Ser–}\overset{*}{\text{Thr}}\text{–Gly–Ser–}}^{33}$	5·50	1·41	0·86	0·73
α-amylase	$\text{–}\underline{\overset{*}{\text{Ser}}\text{–Glu–Asp–Gly}}^{34}$	2·38	1·28	0·96	0·65

* denote the site of attachment of sugar residue to the amino acid.

[b] numbers on the top of the amino acid sequence denote the references.

[c] the amino acids that are involved in a β-turn are underlined.

also been obtained in other sequences containing Asn-sugar linkage, *i.e.*, Thr-Val-Asn(CHO)-Thr ($p_t = 1·65 \times 10^{-4}$; $\langle P_t\rangle = 1·07$, $\langle P_\beta\rangle = 1·14$, $\langle P_\alpha\rangle = 0·88$); and Pro-Lys-Asn(CHO)-Ile ($p_t = 2·72 \times 10^{-4}$; $\langle P_t\rangle = 1·11$, $\langle P_\beta\rangle = 0·90$ and $\langle P_\alpha\rangle = 0·85$) in hormones[5], Ser-Arg-Asn(CHO)-Leu ($p_t = 3·68 \times 10^{-4}$; $\langle P_t\rangle = 1·13$, $\langle P_\beta\rangle = 0·87$, $\langle P_\alpha\rangle = 0·91$) and Arg-Arg-Asn(CHO)-Met ($p_t = 1·69 \times 10^{-4}$; $\langle P_t\rangle = 1·04$, $\langle P_\beta\rangle = 1·03$, $\langle P_\alpha\rangle = 0·88$) in Ribonucleases[5,11–16], Asn (CHO)-Gln-Thr-Gly ($p_t = 2·32 \times 10^{-4}$; $\langle P_t\rangle = 1·29$, $\langle P_\beta\rangle = 0·97$, $\langle P_\alpha\rangle = 0·81$) in human Lacto-transferrin[8], Ser-Asn(CHO)-Tyr-Ser ($p_t = 2·31 \times 10^{-4}$; $\langle P_t\rangle = 1·39$, $\langle P_\beta\rangle = 0·85$, $\langle P_\alpha\rangle = 0·73$) in Taka Amylase A[9], ASn(CHO)-LYS-Ser-Asp ($p_t = 1·90 \times 10^{-4}$); $\langle P_t\rangle = 1·33$, $\langle P_\beta\rangle = 0·73$, $\langle P_\alpha\rangle = 0·89$) in human serotransferrin[10], Pro-Ser-Asn (CHO)-Ser ($p_t = 5·79 \times 10^{-4}$; $\langle P_t\rangle = 1·46$, $\langle P_\beta\rangle = 0·68$, $\langle P_\alpha\rangle = 0·73$) in Guinea pig B Ribonuclease[11], Ser-Ser-Asn(CHO)-Ser ($p_t = 3·98 \times 10^{-4}$; $\langle P_t\rangle = 1·49$, $\langle P_\beta\rangle = 0·70$, $\langle P_\alpha\rangle = 0·78$); and Gln-Ser-Asn(CHO)-Ser ($p_t = 3·16 \times 10^{-4}$; $\langle P_t\rangle = 1·35$, $\langle P_\beta\rangle = 0·83$, $\langle P_\alpha\rangle = 0·87$) in porcine Ribonuclease[14], Ser-Ser-Asn(CHO)-Pro ($p_t = 3·84 \times 10^{-4}$; $\langle P_t\rangle = 1·46$, $\langle P_\beta\rangle = 0·68$, $\langle P_\alpha\rangle = 0·73$) in horse Ribonuclease[16], Met-Asn(CHO)-Gly-Thr ($p_t = 1·94 \times 10^{-4}$; $\langle P_t\rangle = 1·24$, $\langle P_\beta\rangle = 1·08$, $\langle P_\alpha\rangle = 0·82$) in Bovine Visual Pigment 500[18], Gly-Glu-Asn(CHO)-Arg ($p_t = 1·59 \times 10^{-4}$; $\langle P_t\rangle = 1·19$, $\langle P_\beta\rangle = 0·90$, $\langle P_\alpha\rangle = 0·66$) in Fibrinogen[19], Asn(CHO)-Arg-Thr-Gly ($p_t = 2·89 \times 10^{-4}$; $\langle P_t\rangle = 1·30$, $\langle P_\beta\rangle = 0·72$, $\langle P_\alpha\rangle = 0·89$) in hen ovotransferrin[20], Ser-Asn (CHO)-Gly-Ser ($p_t = 3·93 \times 10^{-4}$; $\langle P_t\rangle = 1·51$, $\langle P_\beta\rangle = 0·71$, $\langle P_\alpha\rangle = 0·73$); Asn(CHO)-Asp-Ser-Arg (p_t

TABLE III

$\langle P_t\rangle$, $\langle P_\beta\rangle$, $\langle P_\alpha\rangle$ and p_t values of glycopeptides at the site of sugar linkage to Threonine residue

Glycoprotein	Amino acid sequence at the site of sugar linkage	$p_t \times 10^4$	$\langle P_t\rangle$	$\langle P_\beta\rangle$	$\langle P_\alpha\rangle$
Rabbit Immunoglobulin G[b,c]	–Ser–Lys–Pro–$\overset{*}{\mathrm{Thr}}$–Cys–Pro–Pro[35]	1·83	1·20	0·94	0·69
Phospholipase A (honey bee)[a]	–Tyr–Pro–Gly–$\overset{*}{\mathrm{Thr}}$–Leu–Trp–Cys[25]	7·05	1·27	0·83	0·49
Cow χ_A Casein[a]	–Pro–$\overset{*}{\mathrm{Thr}}$–Thr–$\overset{*}{\mathrm{Thr}}$–Pro–Thr–Thr–[36]	1·83	1·19	1·06	0·76
		2·84	1·19	1·06	0·76
	–Pro–$\overset{*}{\mathrm{Ser}}$–Ser–Ser–Pro	3·49	1·44	0·70	0·74
Sheep χ_A Casein[a]	–His–Ser–$\overset{*}{\mathrm{Thr}}$–Pro–$\overset{*}{\mathrm{Thr}}$–$\overset{*}{\mathrm{Thr}}$–Glu– –Ala–Val[37]	2·84	1·19	1·06	0·76
Alanine Apolipoprotein (apoC–III)	–Val–Arg–Pro–$\overset{*}{\mathrm{Thr}}$–Ser–Ala–Val[38]	2·10	1·23	0·86	0·75
Encephalitogenic Al protein	–Thr–Pro–Arg–$\overset{*}{\mathrm{Thr}}$–Pro–Pro–Pro[39]	2·54	1·14	0·98	0·76

* denote the site of attachment of sugar residue to the amino acid.

[a] denote the site of sugar linkage is not certain.

[b] numbers on the top of the amino acid sequence denote the references.

[c] the amino acids that are involved in a β-turn are underlined.

TABLE IV

$\langle P_t\rangle$, $\langle P_\beta\rangle$, $\langle P_\alpha\rangle$ and p_t values of glycopeptides at the site of sugar linkage to aspartic acid

Glycoprotein	Amino acid sequence at the site of sugar linkage	$p_t \times 10^4$	$\langle P_t\rangle$	$\langle P_\beta\rangle$	$\langle P_\alpha\rangle$
F_c region of Rabbit heavy chain[bc]	–Gln–Gln–Phe–$\overset{*}{\mathrm{Asp}}$–Ser–Thr–Ile[40]	1·92	1·14	1·05	0·90
IgG heavy chain (man baboon, monkey, cow, pig, dog and cat)	--Glx–Glx–Phe–$\overset{*}{\mathrm{Asp}}$–Ser–Thr–Tyr[41]	2·98	1·26	1·00	0·80
	–Gly–Glx–Phe–$\overset{*}{\mathrm{Asp}}$–Ser–Thr–Ile	1·92	1·14	1·08	0·90
Thrombin B chain (bovine)	–Phe–Lys–$\overset{*}{\mathrm{Asp}}$–Pro–Thr–Val[5]	2·01	1·08	1·07	0·88
Protein CRA γ_1	–Asp–Ile–Phe–$\overset{*}{\mathrm{Asp}}$–Asp–Arg–Thr[42]	1·91	1·20	0·89	0·93

* denote site of attachment of sugar residue to the amino acid.

[b] numbers on the top of the amino acid sequence denote the references.

[c] the amino acids that are involved in a β-turn are underlined.

$= 2{\cdot}57 \times 10^{-4}$; $\langle P_t\rangle = 1{\cdot}34$, $\langle P_\beta\rangle = 0{\cdot}77$, $\langle P_\alpha\rangle = 0{\cdot}82$), and Asn(CHO)-Asp-Ser-Cys ($p_t = 2{\cdot}35 \times 10^{-4}$; $\langle P_t\rangle = 1{\cdot}34$, $\langle P_\beta\rangle = 0{\cdot}82$, $\langle P_\alpha\rangle = 0{\cdot}87$) in Fetuin glycopeptides[21], Gly-Pro-Asn(CHO)-Gln ($p_t = 8{\cdot}49 \times 10^{-4}$; $\langle P_t\rangle = 1{\cdot}34$, $\langle P_\beta\rangle = 0{\cdot}83$, $\langle P_\alpha\rangle = 0{\cdot}76$) in human parotid Glycoprotein[22]; Ser-Pro-Asn(CHO)-Ala ($p_t = 5{\cdot}58 \times 10^{-4}$; $\langle P_t\rangle = 1{\cdot}28$, $\langle P_\beta\rangle = 0{\cdot}74$, $\langle P_\alpha\rangle = 0{\cdot}89$); Asn(CHO)-Thr-Thr-Tyr ($p_t = 2{\cdot}44 \times 10^{-4}$; $\langle P_t\rangle = 1{\cdot}22$, $\langle P_\beta\rangle = 1{\cdot}09$, $\langle P_\alpha\rangle = 0{\cdot}75$), Asn(CHO)-Arg-Ser-Ser ($p_t = 3{\cdot}83 \times 10^{-4}$; $\langle P_t\rangle = 1{\cdot}36$, $\langle P_\beta\rangle = 0{\cdot}78$, $(P_\alpha) = 0{\cdot}78$), Asn(CHO)-Ser-Thr-Gln ($p_t = 2{\cdot}20 \times 10^{-4}$; $\langle P_t\rangle = 1{\cdot}27$, $\langle P_\beta\rangle = 0{\cdot}95$, $\langle P_\alpha\rangle = 0{\cdot}88$), Asn(CHO)-Thr-Thr-Ser ($p_t = 2{\cdot}55 \times 10^{-4}$; $\langle P_t\rangle = 1{\cdot}32$, $\langle P_\beta\rangle = 0{\cdot}94$ $\langle P_\alpha\rangle = 0{\cdot}79$) and Tyr-Asn(CHO)-Asn-Ser ($p_t = 3{\cdot}45 \times 10^{-4}$; $\langle P_t\rangle = 1{\cdot}43$, $\langle P_\beta\rangle = 0{\cdot}83$, $\langle P_\alpha\rangle = 0{\cdot}72$) in Horse radish Peroxidase[23], Asp-Arg-

Ser Asn(CHO) ($p_t = 2{\cdot}98 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}34$, $\langle P_\beta \rangle = 0{\cdot}77$ $\langle P_\alpha \rangle = 0{\cdot}82$) in Posterior Pituitary (Pig) Glycopeptides[24], Thr-Pro-Asn(CHO)-Lys ($p_t = 7{\cdot}10 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}24$, $\langle P_\beta \rangle = \langle P_\alpha \rangle = 0{\cdot}80$); Asn(CHO)-Ser-Ser-Tyr ($p_t = 3{\cdot}96 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}39$, $\langle P_\beta \rangle = 0{\cdot}85$, $\langle P_\alpha \rangle = 0{\cdot}73$) in α 1 acid glycoprotein[26]; Gly-Ser-Asn (CHO)-Met ($p_t = 3{\cdot}34 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}32$, $\langle P_\beta \rangle = 0{\cdot}96$ $\langle P_\alpha \rangle = 0{\cdot}81$) in Egg White Avidin[27]; Asn(CHO)-Lys-Thr-Ser ($p_t = 1{\cdot}99 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}27$, $\langle P_\beta \rangle = 0{\cdot}83$, $\langle P_\alpha \rangle = 0{\cdot}85$) in γ chain of human fibrinogen[28] and Asn(CHO)-Thr-Gly-Val ($p_t = 1{\cdot}94 \times 10^{-4}$; $\langle P_t \rangle = 1{\cdot}17$, $\langle P_\beta \rangle = 1{\cdot}08$, $\langle P_\alpha \rangle = 0{\cdot}81$) in Mucor Micher Protease[29]. When this manuscript was completed Aubert *et al.*[43] (1976) from similar analysis of 21 glycoproteins pointed out that 19 out of 28 asparagine-sugar linkages and all the 9 Ser/Thr-sugar linkages assume β-turns. On the other hand the present study based on similar analysis of about 55 glycoproteins predicts that 59 out of 61 asparagine-sugar linkages and all the 21 Ser/Thr-sugar linkages are involved in β-turns, thus showing some discrepancy about the conformation of the protein segment around the Asn-sugar linkages. This may be due to the fact that the Aubert *et al.*[43] (1976) have used original values of Chou and Fasman[2,3] for predicting the β-turns, whereas in the present study the revised values of Chou and Fasman[4] have been made use of. The 2 exceptions in the case of Asn-sugar linkages may be in the disordered or β-sheet regions. The attachment of carbohydrate to protein is known to be post-ribosomal[44] and the sugar linkage is on the surface of the glycoproteins[45-47]. It may be considered that the bend may facilitate the exposure of the side chain of the amino acid for the enzyme to mediate glycosilation.

The amino acid residues occurring in the region around the sugar linkage may be broadly classified into β-turn promoters whose $P_t > 1{\cdot}0$ and non-β-turn promoters whose $P_t < 1{\cdot}0$. In the sequence Asn-X-Ser/Thr, if X is a β-turn promoter such as Gly, Asn, Ser, Pro, and Asp, the triplet has enough potential to form a β-turn, for three of the four residues required to form a β-turn, are β-turn promoters. On the other hand, if X is a non-β-turn promoter residue, the following residue after the triplet sequence Asn-X-Ser/Thr or the two or one of the two preceding residues of this triplet should be β-turn promoters, to satisfy the condition that at least three out of four residues should be β-turn promoters[4] in order to form a bend. It seems that if X is not a β-turn promoter (Ala, Leu, Val, Gln, etc.) the nature of the preceding or following residues to the sequence Asn-X-Ser (Thr) also appear to be important to facilitate the attachment of sugar to the protein chain. It is interesting to note that in sequences Asn-X-Ser(Thr) where X is not a β-turn promoter (Ala, Leu, Lys, etc.) depending upon the nature of the preceding residues, the hydroxy amino acid Ser or Thr in the above sequence may or may not be involved in the formation of a β-turn. It is also interesting to note that in proteins which contain Ser(CHO)-X-Y and Thr(CHO)-X-Y sequences (Tables II and III) the amino acid residue preceding the above sequences in the protein is generally either pro or gly (the β-turn promoters). Table IV shows (though the examples are few) that in Asp linked carbohydrates, the β-turn always begins with Asp and all the amino acids in the triplet sequence Asp(CHO)-X-Thr/Ser are involved in the β-turn.

Ser/Thr(CHO)-X-Y-Pro was suggested earlier as 'code sequence'[36] similar to Asn(CHO)-X-Ser/Thr for glycopeptides containing the N-glucosamine linkage. Tables II and III indicate that in the above sequence Ser/Thr(CHO)X–Y–Z, Z need not necessarily be a pro in disagreement with the earlier suggestions.

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1. Marshall, R. D., *Annu. Rev. Biochem.*, 1972, **41**, 673.
2. Chou, Y. P. and Fasman, G. D., *Biochemistry*, 1974, **13**, 211.
3. — and —, *Ibid.*, 1974, **13**, 222.
4. — and —, *J. Mol. Biol.*, 1975, **96**, 29.
5. *Hand Book of Biochemistry and Molecular Biology*, Vols. II and III (ed. Fasman, G. D.), CRC Press, Cleveland, 1975.
6. Thomas, H. Plummer Jr. and Louis Sarda, *J. Biol. Chem.*, 1973, **248**, 7865.
7. Rydess, L. and Baker, D., *Eur. J. Biochem.*, 1974, **44**, 171.
8. Spik, G., Vandersyppe, R., Montreuil, J. Tetaert, D. and Hass, K. K., *FEBS Letters*, 1974, **38**, 213.
9. Isemura, S. and Ikenaka, T., *J. Biochem.*, 1973, **74**, 1.
10. Charet, P., Tetaert, D., Hass, K. K. and Montreuil J.. *C.R. Hebd*, *Seances Acad. Sci. Paris*, Ser. D., 1973, **276**, 1629.
11. Vanden Berg, A. and Beintema, J. J., *Nature*, 1975, **253**, 207.
12. Wim Gaastra, Gerda Groen, Gjalt W. Welling and Beintema, J. J., *FEBS Letters*, 1974, **41**, 227.
13. Gita Leijenaar-Van den Berg and Beintema, J. J., *Ibid.*, 1975, **56**, 101.
14. Jackson, R. L. and Hirs, C. H. W., *J. Biol. Chem.*, 1970, **245**, 637.

15. Henk Zwiers, Albert, J. Scheffer and Beintema, J. J., *Eur. J. Biochem.*, 1973, 36, 569.

16. Welling, G. W., Groen, G., Gabel, D., Gaastra, W. and Beintema, J. J., *FEBS Letters*, 1974, **40**, 134.

17. Shainkin Ruth and Gertrude E. Perlmann, *J. Biol. Chem.*, 1971, **246**, 2278.

18. Joram Heller and Mariane, A. Lawrence, *Biochemistry*, 1970, **9**, 864.

19. Mester, L., Moczar, E. and Szabados, L. C. R., *Hebd. Seances Acad. Sci.* (*Paris*), 1968, 64, 683.

20. Barry Kingston, I. and John Williams, *Biochem. J.*, 1975, **147**, 463.

21. Begbie, R., *Biochem. Biophys. Acta*, 1974, **371**, 549.

22. Pierre Degand, Arnold Boersma, Philippe Roussel, Colotte Richet and Gerard Biserte, *FEBS Letters*, 1975, **54**, 189.

23. Welinder, K. G. and Smillie, *Can. J. Biochem.*, 1972, **50**, 63.

24. Holwerda, D. A., *Eur. J. Biochem.*, 1972, **28**, 340.

25. Shipoline, R. A., Callewaert, G. L., Cottrell, R. C. and Vernon, C. A., *FEBS Letters*, 1971, **17**, 39.

26. Karl Schmid, Hugo Kaufmann, Satako Isemura, Francine Bauer, Jungi Emura, Toshiko Motoyama, Masatsune Ishiguro and Shigeru Nanno, *Biochemistry*, 1973, **12**, 2711.

27. Delange, R. J. and Tung-Shiuh Huang, *J. Biol. Chem.*, 1971, **246**, 698.

28. Sadaaki Iwanaga, Birger Blomback, Nils, J. Grondahl, Birgit Hessel and Per Wallen, *Biochim. Biophys. Acta*, 1968, **160**, 280.

29. Rickert, W. S. and Mcbride-Warren, P. A., *Ibid.*, 1974, **336**, 437.

30. Frangione Blas and Carlota Wolfenstein-Todel, *Proc. Natl. Acad. Sci. U.S.A.*, 1972, **69**, 3673.

31. Jacques Baenziger and Stuart Kornfield, *J. Biol, Chem.*, 1974, **249**, 7270.

32. Frangione, B. and Milstein, C., *Nature*, 1969, **224**, 597.

33. Tsuneo Ozeki and Zensaku Yosizawa, *Arch. Biochem. Biophys.*, 1971, **142**, 177.

34. Tsugita, A. and Akabori, B., *J. Biochemistry* (*Tokyo*), 1959, **46**, 695.

35. Fanger, M. W. and Smyth, D. G., *Biochem. J.*, 1972, **127**, 767.

36. Jolles, J., Schentgen, F., Alais, C., Fiat, A. M. and Jolles, P., *Helv. Chim. Acta*, 1972, **55**, 2872.

37. Jacqueline Jolles, Anne-Marie Fiat, Francoise Schoentgen, Charles Alais and Pierre Jolles, *Biochem. Biophys. Acta*, 1974, **365**, 335.

38. Bryan Brewar, H. Jr., Richard Shulman, Peter Herbert, Rosemary Ronan and Katherine Wehrly, *J. Biol. Chem.*, 1974, **249**, 4975.

39. Hagopian, A., Whitehead, J. S. and Westall, F. C., *Fed. Proc.*, 1971, **30**, 1186.

40. Robert, L. Hill, Robert Delaney, Robert, E. Fellows Jr. and Harold E. Lebovitz, *Proc. Natl. Acad. Sci. U.S.A.*, 1966, **56**, 1762.

41. Howell, J. W., Hood, L. and Sanders, *J. Mol. Biol.*, 1967, **30**, 555.

42. Sheldon M. Cooper, Edward C. Franklin and Blas Frangione, *Science*, 1972, **176**, 187.

43. Jean Pierre Aubert, Gerard Biserte and Marie H. Loucheux Lefebvre, *Arch. Biochem. Biophys.*, 1976, **175**, 410.

44. Spiro, R. G., *New Engl. J. Med.*, 1969, **281**. 991, 1043.

45. Hunt, L. T. and Dayhoff, M. O., *Biochim. Biophys. Res. Commun.*, 1970, **39**, 757.

46. Dickerson, R. E. and Geis, I., *The Structure and Action of Proteins*, Harper and Row, New York, 1969, p. 19.

47. Shotton, D. M. and Hartley, B. S., *Nature*, 1970, **225**, 802.

FIRST ALL INDIA SYMPOSIUM ON METABOLISM AND DISPOSITION OF XENOBIOTICS

The First All India Symposium on Metabolism and Disposition of Xenobiotics, will be held at Marathwada University, Aurangabad 431002, during 22–44 November 1977. Papers are invited in all aspects of studies in Biological systems related to the metabolism and disposition of exogenous chemicals, toxins, chemotherapeutic agents, carcinogens, herbicides, pesticides, etc. Abstracts of 200 words or less are due by **July 31, 1977** and manuscripts by **August 31, 1977.**

For details contact : **Prof. S. S. Pawar, Biochemistry Division, Chemistry Department, Marathwada University, Aurangabad 431002.**

Cu(II), Ni(II) AND Co(II) COMPLEXES OF DISALICYLALDIMINE OXAMIDE AND N, N′-Di (O-HYDROXYACETOPHENONE IMINE) OXAMIDE, THE SCHIFF BASES DERIVED FROM OXALDIHYDRAZIDE AND SALICYLALDEHYDE AND O-HYDROXYACETOPHENONE

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ABSTRACT

Cu(II), Ni(II) and Co(II) complexes of the title ligands have been synthesized and characterized by analytical, spectroscopic and magnetic moment data. The complexes are coloured and insoluble in common organic solvents and decompose above 250° C. Cu(II) complexes are planar or pseudo-octahedral while Ni(II) and Co(II) complexes have octahedral stereochemistry. Some of them show subnormal magnetic moments. The ligands coordinate to Cu(II) in enol form and Ni(II) and Co(II) in the keto form.

INTRODUCTION

RECENTLY, a number of publications have appeared on the transition metal complexes of Schiff bases derived from monoacylhydrazines[1-5]. These bases act either as a bidentate or tridentate ligands and some of their compounds show subnormal magnetic moments. The dihydrazides having two-CONH NH$_2$ groups have been reported to coordinate through $\rangle$NH[6]. Since no previous work on the Schiff bases derived from the organic acid dihydrazides has been reported, the present paper, therefore, describes Cu(II), Ni(II) and Co(II) complexes of the title ligands.

MATERIALS AND METHODS

Preparation of the ligands.—Oxaldihydrazide[7], m.p. 234° (Lit. 232°), was prepared by the reaction of diethyloxalate diluted with ethanol and hydrazine hydrate in 1 : 2 molar ratio. The product was filtered and washed with alcohol. The ligands H_2L and H_2J were prepared by mixing dilute ethanolic solution of oxaldihydrazide and salicylaldehyde and O-hydroxyacetophenone in 1 : 2 molar ratio. The products were filtered, washed with hot water to free from oxaldihydrazide and finally with ethanol and dried at 110°.

H_2L, m.p. >250° Found (Calcd. %) C = 58·30 (58·99); H = 4·20 (4·29): N = 16083 (16·50).

H_3J, m.p. >250° Found (Calcd. %) C = 60·27 (61·02); H = 5·0(5·10): N = 15·50 (15·82)

Preparation of the complexes.—Since both the ligands are insoluble in water and very slightly soluble in ethanol, the complexes were prepared by taking metal : ligand (8 : 1) mixture in water or ethanol and stirring at room temperature (30°) or above (70°) for 24–36 hr. The products were filtered, washed with water or alcohol and dried at room temperature over anhydrous calcium chloride.

Analytical procedures and instruments used were same as reported earlier[5]. The data are summarized in Tables I and II.

RESULTS AND DISCUSSION

Cu(II) reacts with H_2L both in alcoholic and aqueous media while Ni(II) and Co(II) fail to react with it. H_2J, however, reacts with Cu(II), Ni(II) and Co(II) chloride in alcoholic medium but only Cu(II) complex could be isolated from aqueous medium.

All the complexes have 1 : 1 stoichiometry. The complexes prepared in alcoholic medium have one chloride ion present while the complexes prepared in aqueous medium are free from the anion. All the complexes are coloured and insoluble in common organic solvents and decompose or melt with decomposition above 250° C. Loss of weight at 110° corresponds to number of water molecules in the lattice structure or weakly coordinated water molecules. The solubility and high decomposition temperature indicate the polymeric nature of complexes in solid state[8].

Electronic Spectra

The chromophores $\rangle C{=}O$, $\rangle C{=}N-$ in conjugation with benzene nucleus, shift the primary and secondary bands of benzene at 204 and 256 nm respectively to longer wavelength[9]. In H_2L, the 204 nm band appears at 200 nm and the other band at 256 nm shifts to 295 nm. A band characteristics of the salicylaldehydato group[10] appears at 365 nm. In H_2J, the two bands appear at 225 and 310 nm respectively.

In metal complexes, the absorption bands are so much red shifted that the characteristic bands of salicylaldehyde group appears as high as 400 nm. Such a large red shift may be due to metal-ligand bonds. The Cu(II) complexes (nos. *a, b, c* and *f*), in addition to the ligand bands, show bands around 705, 685, 625 and 570 nm respectively. The octahedral complexes of Cu(II) show bands around 800 nm[11] in some olive green square planar Cu(II) chelates[12]. The appearance of a high energy band around 400 nm or 450 nm

TABLE I

Analytical, electronic and magnetic moment data of disalicylaldimine oxamide (H_2L) *and N, N′- di (O-hydroxyacetophenone imine) oxamide* (H_2J) *complexes of Cu(II), Ni(II) and Co(II)*

Sl. No.	Complex and Colour	Melting point/ decomposition point	Analysis (%)				Magnetic moment/ μ_{eff} B.M.	Electronic bands/ λ_{max} nm
			Metal	Nitrogen	Ligand	Anion		
(*a*)	Cu (HL) Cl·$2H_2O$ Darkgreen	>250 m	14·2 (13·8)	12·7 (12·2)	70·2 (70·8)	8·0 (7·7)	1·9	255, 345, 463, 705
(*b*)	CuL·$2H_2O$ Darkgreen	>250 m	14·7 (15·0)	13·2 (13·2)	77·0 (76·5)	..	1·8	220, 255, 340, 450, 475, 685
(*c*)	Cu (HJ) Cl·$2H_2O$ Darkgreen	>250 m	13·0 (13·5)	11· (11·9)	75·8 (75·1)	7·7 (7·6)	2·0	245, 305, 400, 625
(*d*)	Ni (HJ) Cl·$2H_2O$ Green	>250 m, d	13·5 (13·1)	12·0 (11·6)	73·8 (73·0)	6·9 (7·3)	3·0	225, 290, 610, 1000
(*e*)	CO (HJ) Cl·$2H_2O$ Purple	>250 m, d	12·8 (13·1)	11·3 (11·6)	73·2 (73·0)	7·2 (7·3)	5·1	230, 310, 525, 660, 687
(*f*)	CuJ·$2H_2O$ Darkgreen	>250 m	13·8 (14·1)	12·0 (12·4)	77·0 (77·9)	..	1·73	245, 325, 390, 570

m = melts. m, d = melts with decomposition.

TABLE II

Some important infrared frequencies of H_2L *and* H_2J *and their metal complexes (Frequencies in* cm^{-1}*)*

Sl. No.	Complexes	ν(OH) + ν(NH)	ν(C=O)	ν(C=N)	ν(NCO)	Amide III	ν(C—O)	ν(N—N)
(*a*)	H_2L	3285 3210	1665	1600	..	1372	1255	1033
(*a*)	Cu (HL) Cl·$2H_2O$	3670 3373	..	1598	1546	1380	1275 1250	1050
(*b*)	CuL·$2H_2O$	3416	..	1608	1542 1533 1525	1375	1250	1037
	H_2J	3300	1684 1646	1604	..	1372	1242 1233	1020
(*c*)	Cu (HJ) Cl·$2H_2O$	3526 3450 3350	..	1595 1580	1540 1512	1370	1260 1235	1020
(*d*)	Ni (HJ) Cl·$2H_2O$	3516 3416 3296 3150	1675 1644	1600	..	1375	1258 1242 1220	1020
(*e*)	CO (HJ) Cl·$2H_2O$	3440 3275	1650	1605	..	1375	1245 1235	1030
(*f*)	CuJ·$2H_2O$	3605 3365 3300	..	1590	1540 1520	1360	1275 1242	1010

in some Cu(II) complexes has been a subject-matter of considerable discussion and it has been related to the presence of binuclear structures of Cu(II) complexes. Keeping the above facts and the observed magnetic moments in view, it is plausible to assign a molecularly associated or oxobridged structures for the Cu(II) complexes (nos. *b*, *c* and *f*) while in the complex (no. *a*) the band observed at 705 nm indicates it to have a pseudo-octahedral structure. The octahedral Ni(II) complexes[13] show three bands due to ${}^3A_{2g} \rightarrow {}^3T_{2g}$ (F), ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (F) and ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (P) transitions. In the Ni(II) complex (no. *d*), the first two bands are observed at 1000, 610 nm respectively confirming its octahedral structure. In octahedral Co(II) complexes several transitions are expected but all of them are seldom observed[11]. The colour and absorption bands of Co(II) complex (no. *e*) indicate an octahedral stereochemistry.

Magnetic Moment

Cu(II) complexes (nos. *a* and *c*) synthesized in alcoholic medium are normal paramagnetic corresponding to one unpaired electron. The Cu(II) complexes (nos. *b* and *f*) prepared in aqueous medium have magnetic moment value slightly less than the value for one unpaired electron. The low magnetic moments may be due to some M–M interactions *via* oxo-bridges. The magnetic moment values of Ni(II) and Co(II) complexes correspond to two and three electrons respectively in octahedral environment.

Infrared Spectra

Coordination of monoacylhydrazones to metal ions through $\rangle C{=}O$ and $\rangle C{=}N$ lowers $\nu(C{=}O)$ and $\nu(C{=}N)$[13]. When these ligands undergo enolization, the $\rangle C{=}O$ and $\rangle NH$ groups are destroyed giving rise to $-C(OH):N-$ group in which $\nu(OH)$, $\nu(C{-}O)$, and $\nu(C{=}N{-})$ should appear. Reaction with metal ions in enol form results in $-N{=}C{-}O$[14] and $\rangle C{=}N{-}N{=}C\langle$[1-3] group vibrations.

In H_2L and H_2J, the bands in region 3000–3300 cm^{-1} [$\nu(OH) + \nu(NH)$] and 1700–1650 cm^{-1} [$\nu(C{=}O)$] indicate that the ligands exist primarily in the keto form. These spectra indicate the presence of strong intramolecular H-bonding in the molecule. In Cu(II) complexes broad band appears in the region 3300–3600 cm^{-1} indicating the presence of free —OH group of either phenolic —OH or lattice water molecules. The $\nu(C{=}O)$ stretching frequency originally present in the ligands has disappeared in these complexes ruling out the possibility of coordination through $\rangle C{=}O$ group. A very sharp and intense band at 1600 cm^{-1} is observed indicating the presence of coordinated azine group $\rangle C{=}N{-}N{=}C\langle$ while the $NC\bar{O}$ group vibrations observed at about 1540 cm^{-1} confirm the enolization process. The bands at 1255 cm^{-1} in H_2L and 1242 and 1233 cm^{-1} in H_2J, assigned to $\nu(C{-}O)$, shift to higher frequencies on chelation. It appears the ligand chelates with Cu(II) in enol form.

The i.r. spectra of Ni(II) and Co(II) complexes (nos. *d* and *e*) show a broad band in the region 3500–3300 cm^{-1} and a sharp and strong peak around 3300 cm^{-1} which may be assigned to free $\nu(OH)$ of ligand or lattice water and $\nu(NH)$ respectively. The $\nu(C{=}O)$ stretching frequency originally present at 1684, 1646 cm^{-1} shifts to lower frequency by 10–30 cm^{-1} indicating coordination of the $\rangle C{=}O$ group. Amide II band in H_2J appears at 1560 cm^{-1} but splits up in complexes and shows positive as well as negative shift. The decrease in frequency of amide II may be related to coordination of $\rangle C{=}O$ group. Amide (III) band at 1372 cm^{-1} and $\nu(C{-}O)$ at 1242 and 1233 cm^{-1} in H_2J shift to higher frequencies in metal complexes.

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1. Narang, K. K. and Aggarwal, A., *Inorg. Chim. Acta*, 1974, 9, 137; *Indian J. Chem.*, 1975, 13, 1072.
2. Iskander, M. F., El-Aggan, A. M., Refaat, L. S. and Elsayed, L., *Inorg. Chim. Acta*, 1975, 14, 167.
3. —, Elsayed, L. and Lasheen, M. A., *Ibid.*, 1976, 16, 147.
4. Biradar, N. S. and Havinale, B. R., *Ibid.*, 1976, 17, 157.
5. Narang, K. K. and Lal, R. A., *Indian J. Chem.*, 1976, 14 A, 442.
6. Dutta Ahmad, A., Mandal, P. K. and Roy Chaudhary, N., *J. Inorg. Nucl. Chem.*, 1966, 28, 2951.
7. Prager, B. and Jacobson, P., *Beilstein Handbuch Der Organischen Chemie*, 1920, 2, 559.
8. Ginsberg, A. P., Martin, R. L. and Sherwood, R. C., *Inorg. Chem.*, 1968, 7, 932.
9. Jaffé, H. H. and Orchin Milton, *Theory and Applications of Ultraviolet Spectroscopy*, John Wiley, New York, 1967, p. 164.
10. Rohatgi K. K. and Sengupta, S. K., *J. Inorg. Nucl. Chem.*, 1972, 34, 3061.
11. Dunn, T. M., "The visible and ultraviolet spectra of complex compounds," In: *Modern Coordination Chemistry*, Eds. J. Lewis and R. G. Wilkins, Interscience, New York, N.Y., 1960.
12. Sacconi, L. and Ciampolini, M., *J. Chem. Soc.*, 1964, p. 276.
13. Issa, R. M., El-Shazly, M. F. and Iskander, M. F., *Z. Anorg. Allg. Chem.*, 1967, 98, 354.
14. Mashima, M., *Bull. Chem. Soc. Japan*, 1962, 35, 338.

ANALYSIS OF THE GRAVITY EFFECT DUE TO HORIZONTAL CIRCULAR CYLINDER WITH VARIABLE DENSITY

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ABSTRACT

Fourier transform of the gravity anomaly of a horizontal circular cylinder with density contrast varying linearly with depth has been derived. The Fourier spectrum has been analysed to obtain the parameters, *viz.*, depth to the centre of the cylinder, radius of the cylinder and the rate of change of density contrast. The validity of the method has been tested on a synthetic model.

INTRODUCTION

In gravity interpretation, the geometrical shapes of the anomaly producing bodies are assumed and the density contrast is usually considered to be uniform. But very frequently we come across situations wherein the density contrast increases with depth as it is evident from the seismic studies[1,2]. This variation in density contrast is attributed to the compaction of the sedimentary strata that lie over the basement rocks.

Some authors have made an attempt to interpret the gravity anomaly due to bodies with non-uniform density. For instance Radhakrishna Murthy[3] derived the gravity effect due to a horizontal circular cylinder with the density contrast varying linearly with depth. However, his method of analysis is cumbersome and does not yield all the parameters.

In this paper, an elegant interpretation technique is presented to analyse the gravity anomaly due to a horizontal circular cylinder with density contrast linearly increasing with depth, by assuming the density contrast at the apex of the body.

Fourier Transform of the Gravity Anomaly

Consider the horizontal circular cylinder whose geometrical configuration is given in Fig. 1. We assume the density contrast at the apex of the cylinder as σ. Let the rate of change of density contrast varying linearly with depth be a. The gravity anomaly of the cylinder in the present case is given by[3]

$$g(x) = A\frac{Z}{(x^2 + z^2)} - B\frac{z^2 - x^2}{(x^2 + z^2)^2} \tag{1}$$

where

$$A = 2\Pi\gamma R^2(\sigma + aR) \tag{2}$$

and

$$B = \frac{\Pi\gamma a R^4}{2} \tag{3}$$

and γ being the universal gravitational constant.

The Fourier transform of the function g(x) can be written as

$$FT(\omega) = \int_{-\infty}^{\infty} g(x)e^{-i\omega x}\,dx \tag{4}$$

where ω is the frequency.

Since the gravity function for a horizontal circular cylinder is an even function only the cosine transform need be considered. Substituting for g(x) from equation (1) and evaluating the integral we get

$$FT(\omega) = \Pi e^{-\omega z}(A - B\omega). \tag{5}$$

From equation (5) it is evident that the components of Fourier spectrum mainly depend on $\exp(-\omega z)$ and the parameters can be evaluated only at lower frequency ranges[4].

The intercept by $\omega = 0$ in the distribution of $FT(\omega)$ *versus* ω will yield

$$FT(0) = \Pi A.$$

Thus

$$A = \frac{FT(0)}{\Pi}. \tag{6}$$

If $FT(\omega)$ vanishes for $\omega = \omega_1$ then we may write

$$\Pi e^{-\omega z}(A - B\omega_1) = 0.$$

And hence we get

$$B = \frac{A}{\omega_1}. \tag{7}$$

Since A and B are already known from equations (6) and (7), by calculating $FT(\omega)$ at a non-zero frequency say $\omega = \bar{\omega}$ then we can determine the depth to the centre of the cylinder

$$Z = \frac{1}{\bar{\omega}}\left[\log \Pi(A - B\bar{\omega}) - \log FT(\bar{\omega})\right]. \tag{8}$$

By eliminating 'a' from equations (2) and (3), we get the cubic equation in R as

$$R^3 - \frac{A}{2\sigma\Pi\gamma}R + \frac{4B}{2\sigma\Pi\gamma} \cancel{= 0} \tag{9}$$

we apply the well known Cardan's method for solving R from equation (9). Then a is calculated from

$$a = \frac{2B}{R^4}. \qquad (10)$$

Theoretical Example

In usual field practice, we record the gravity effect at discrete points and not as a continuous function. Though it is possible to get a continuous estimate of the function in synthetic model, by viewing from practical applicability we digitize the anomaly. The gravity anomaly given by equation (1) is computed with the values $\sigma = 0{\cdot}2$ gm/c.c.; $a = 0{\cdot}8$ gm/c.c/km; R = 1 km and Z = 2 km at an interval of 0·5 km and shown in Fig. 1. We digitize the anomaly and construct a synthetic model.

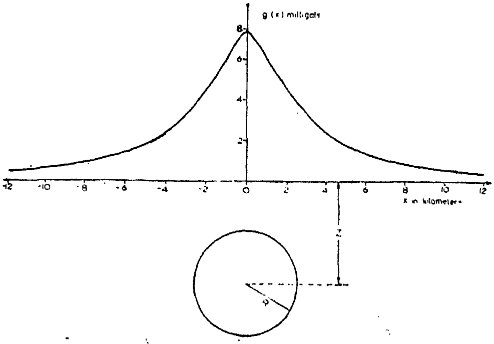

FIG. 1. Gravity anomaly of the horizontal circular cylinder with density contrast varying linearly with depth.

The digitized Fourier transform[5] of this data, with discrete frequencies, is given by

$$\text{DFT}\ (n\omega_0) = \sum_{l=1}^{N} G\,(l.\triangle x) e^{-in\omega_0 l \triangle x} \qquad (11)$$

where $\omega_0 = 2\Pi/N\text{-}\triangle x$ is the fundamental frequency, N is the number of points and $\triangle x$ is the sample interval. The amplitudes at frequencies not higher than the nyquist frequencies (2 Π radians/km) are considered. The graph of DFT $(n\omega_0)$ *versus* $n\omega_0$ is shown in Fig (2). The values of the parameters evaluated from Fig. 2 and using equations (6), (7), (8), (9), and (10), namely $a = 0{\cdot}685$ gm/c.c./km, R = 0·925 km and Z = 2·12 km indicate that they are sufficiently close to the assumed values.

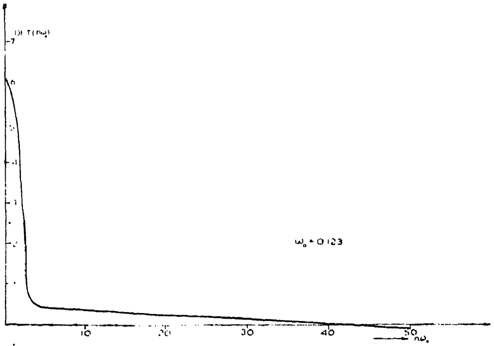

FIG. 2. The graph of DFT $(n\omega_0)$ *versus* $n\omega_0$ of the gravity anomaly.

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1. Skeels, D. C., *Geophysics*, 1947, **12**, 43.
2. Dobrin, M. B., *Introduction to Geophysical Prospecting*, McGraw-Hill Book Company, Inc., New York, 1952.
3. Radhakrishna Murthy, I. V., *Bulettino dell Associazione Geofisica Italina*, 1973, **22** (5/6), 407.
4. Bhimasankaram, V. L. S., Mohan, N. L. and Seshagiri Rao, S. V., "Analysis of gravity effect of two dimensional trapezoidal prism using Fourier transforms," *Geophysical Prospecting* (In press).
5. Cochran, W. T., Cooley, T. W., Favin, D. L., Helms, H. D., Kaenel, R. A., Lang, W. W., Maling, G. C., Nelson, D. E., Rader, C. M., and Welch, P. D., "What is the fast Fourier transform?," *IEEE Transactions on Audio and Electroacoustics*, 1967, **AU–15**, 45.

BINDING IN SINGLET- AND TRIPLET-STATE EXCIMERS

II. Role of Subjacent Orbital Interactions*

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ABSTRACT

Theoretical studies on excimers have invariably been restricted to treating excimers as a four-electron problem and are unsatisfactory both with respect to accounting spectral characteristics, and geometry of snglet/triplet excimer. In a previous paper [*Mol. Phys.* 30, 319 (1975)], we have studied the possible geometries of singlet/triplet excimer, based on the 4-electron problem and concluded that the perfect sandwich structure is the most stable conformation for an excimer. In the present work, an excimer is treated as an $4n$-electron problem ($n = 2, 3$) and the results for naphthalene and anthracene systems indicate that singlet and triplet excimers can have different geometries. The role of subjacent orbitals is also discussed.

1. INTRODUCTION

AN excimer is a dimer, which is associated in an excited electronic state, and dissociative (*i.e.*, would dissociate in absence of external restraints) in its electronic ground state[1]. Collisional interaction between a singlet/triplet excited atom/molecule ($^{1,3}M^*$) and an unexcited atom/molecule ($^{1,3}M$).

$$^{1,3}M^* + {}^{1,3}M \rightleftharpoons {}^{1,3}D^*$$

may yield a singlet/triplet excimer. While much experimental data are available[1-3], a satisfactory theory to account for the spectral characteristics, differences in stability and geometry of singlet/triplet excimer is lacking. Theoretical studies have invariably been restricted to treating an excimer as a 4-electron problem[4-8]. In this work, we report the preliminary results of our efforts to extend 4-electron model to a $4n$-electron model ($n = 2, 3$), which takes into account not only the highest occupied (HOMO) and lowest unoccupied molecular orbitals (LUMO) of each monomer, but also the next highest (occupied) and lower (unoccupied) molecular orbitals—the subjacent orbitals of each monomer. The importance of inclusion of subjacent orbitals has been shown by Berson and Salem[9], in their work on forbidden reactions, in Woodward-Hoffman's sense.

2. METHOD

We use the super-molecular-orbital theory[8], and the method is similar to our earlier work[10] (appendix, for some details on evaluation of integrals).

3. RESULTS AND DISCUSSION

In Fig. 1 is shown the dimer orbitals (in super-molecular-orbital approach) for naphthalene and anthracene excimer, in the $4n$-electron model, where dimer orbital $2k$ and $2k - 1$ belong to k^{th} parentage (of monomer). As regards geometry, we consider two cases :

(i) a perfect sandwich structure (D_{2h} symmetry).
(*ii*) a 'tilted' structure in which long axes of the molecules are parallel and short axes make an angle 'α'.

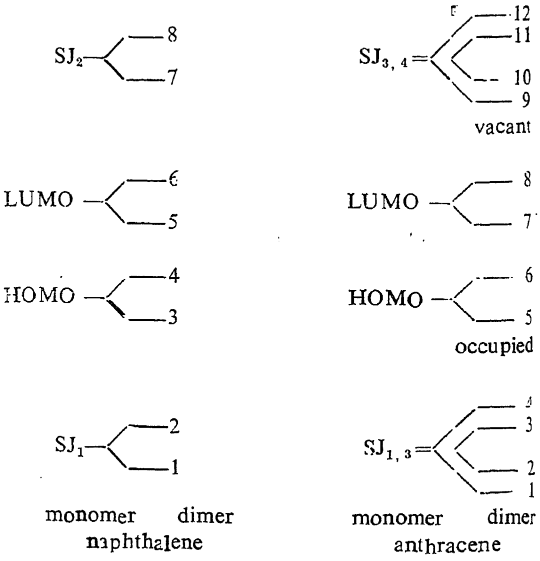

FIG. 1. Numbering (and parentage from monomer) of MO's in excimer of naphthalene and anthracene (schematic).

Note: $2k^{th}$ and $(2k-1)^{th}$ dimer orbitals belongs to k^{th} parentage (of monomer).

Primary motivation in considering these two types only is that, our previous experience[10] suggests that rotated sandwich structure is less stable when compared to sandwich structure. Moreover, Ferguson *et al.*[11] have clearly ruled out the possibility of a rotated structure. Further, we wish to study theoretically, the possibility of singlet and triplet excimers having different structures. It is increasingly felt[12,13] that singlet and triplet excimer may have different steric configurations.

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3.1. *Role of Subjacent Orbitals*

In Table I are given the coefficients for the various configurations [after configuration interaction (CI)] for the lowest excited state, in naphthalene excimer. It is clear that for the singlet state, the subjacent orbitals interaction is important, while for the triplet states, the HOMO/LUMO interaction is important. In Table II is shown the non-zero (and > 0·1) coefficient of the various configurations (after CI) for the lowest excited state of anthracene excimer. It is obvious from a close analysis of the data that in the case of singlet state, HOMO/LUMO interaction is predominant, while for the triplet states, there is some contribution from subjacent orbitals [*i.e.*, transition from HOMO (and subjacent occupied MO) to vacant subjacent orbital]. We feel that in higher polyacenes excimers, say, pyrene, perylene, etc.; the 4-electron model may be sufficient for describing singlet excimers, while transitions from/to subjacent orbitals are important for describing triplet excimers.

3.2. *Stability of Singlet/Triplet Excimer*

Of the several low lying excimer states, we shall be concerned with only the lowest state, σ-state, the emitting state of excimer. To examine the stability of σ-state (singlet/triplet) of an excimer with respect to dissociation into a monomer ground and a monomer singlet/triplet states, we need to examine the differences between the energy. $E_{1,3\sigma}$ of the singlet/triplet σ-state of an excimer and the sum of $E_{1,3p}$, the energy of the singlet/triplet *p*-state and E_M–the energy of ground state of the monomer. The interaction energy is given by

$$E_{1,3\sigma} - E_{1,3p} - E_M = {}^{1,3}\Delta E + y \qquad (1)$$

where

$${}^{1,3}\Delta E = E({}^{1,3}\sigma) - E({}^{1,3}p) \qquad (2)$$

and $E({}^{1,3}\sigma)$ and $E({}^{1,3}p)$ are the energies of singlet/triplet excimer σ, and the monomer *p* bands respectively. Both ${}^{1,3}\Delta E$ and '*y*' are functions of the structural parameters in the dimer structure. In Table III is shown the binding energy of an excimer both in tilted and sandwich conformation

TABLE I

Coefficients of the various configurations, in the lowest excited state of naphthalene excimer. Pivotal distance D = 3·5 Å

Tilt angle α		(*m*, *n*) 4, 5*	4, 7	3, 6	3, 8	2, 5	2, 7	1, 6	1, 8
0°	Singlet	0·0	−0·2940	0·0	−0·3803	0·4764	0·00	0·7362	0·0
	Triplet	0·6061	0·0	0·7836	0·0	0·0	0·0884	0·0	0·1041
40°	Singlet	0·0	−0·3702	0·0	−0·4035	0·5643	0·0	0·6178	0·0
	Triplet	0·6671	0·0	0·7321	0·0	0·0	0·0961	0·0	0·0989
70°	Singlet	0·0	−0·3983	0·0	−0·4197	0·5643	0·0	0·5888	0·0
	Triplet	0·6786	0·0	0·7214	0·0	0·0	0·0972	0·0	0·0981

* (*m*, *n*) indicates transition from *m*th occupied dimer orbital to *n*th vacant orbital. Similar is the behaviour for all D's and tilt angles.

TABLE II

Coefficients of the various configurations, in the lowest excited state of anthracene excimer, for privotal distance D = 3·2 Å

Tilt angle (°)		(*m*, *n*) 6, 7[a]	5, 8	4, 9	3, 10[b]
0	Singlet	0·269	0·955	0·0	0·00
	Triplet	0·506	0·816	0·158	0·215
45	Singlet	0·522	0·837	0·0	0·0
	Triplet	0·619	0·732	0·183	0·202
90	Singlet	0·620	0·764	0·0	0·0
	Triplet	0·651	0·703	0·189	0·198

[a] (*m*, *n*) indicates the transition from *m*th occupied dimer orbital to *n*th vacant orbital.

[b] Only those configurations for which the coefficients are at least 0·1 are included in this table. Similar behaviour is noticed for other distances and tilt angles

TABLE III

Binding energies in naphthalene excimer (in eV)

Tilt angle α (°)	Tilted conformation Pivotal distance D (Å) 3·2 Singlet	 Triplet	 3·5 Singlet	 Triplet	Pivotal distance D (Å)	Sandwich conformation (zero tilt angle) Singlet	 Triplet
0	−0·401	−0·571	−0·707	−1·054	3·3	−0·5483	−0·851
10	−0·622	−0·964	−0·745	−1·123	3·4	−0·6451	−0·973
20	−0·654	−1·038	−0·724	−1·121	3·5	−0·7067	−1·054
30	−0·649	−1·057	−0·699	−1·109	3·6	−0·7432	−1·104
40	−0·639	−1·064	−0·677	−1·098	3·7	−0·7626	−1·134
50	−0·625	−1·060	−0·658	−1·088	3·8	−0·7702	−1·149
60	−0·605	−1·048	−0·630	−1·076	3·9	−0·7703	−1·155
70	−0·573	−1·022	−0·618	−1·061	4·0	−0·7655	−1·154
80	−0·521	−0·975	..	..	4·1	−0·7580	−1·150
90	−0·447	−0·903	..	..			

TABLE IV

Binding energies in anthracene excimer (in eV)

Pivotal distance D (Å) Tilt angle	Tilted conformation 3·2 Singlet	 Triplet	 3·5 Singlet	 Triplet	Pivotal distance D(Å)	Sandwich conformation Singlet	 Triplet
0°	−0·0930	−0·3459	−0·4897	−0·9087	3·2	−0·0930	−0·346
10°	−0·4490	−0·7780	−0·6085	−1·0285	3·4	−0·418	−0·792
20°	−0·4407	−0·8817	−0·5614	−1·0114	3·5	−0·490	−0·909
30°	−0·4321	−0·9051	−0·4930	−0·9770	3·6	−0·526	−0·981
40°	−0·4150	−0·9090	−0·4395	−0·9505	3·7	−0·539	−1·022
50°	−0·3928	−0·9018	−0·3918	−0·9228	3·8	−0·538	−1·043
60°	−0·3558	−0·8817	−0·3635	−0·9125	3·9	−0·527	−1·047
70°	−0·3043	−0·8363	−0·3374	−0·9074	4·0	−0·512	−1·045
80°	−0·2224	−0·7624	−0·2645	−0·8575			
90°	−0·1055	−0·6525	+0·0390	−0·8110			

for different values of pivotal distances for naphthalene excimer. An examination of the data reveals that at lower values of 'D' it is the 'tilted' conformation that is stable while at larger values of 'D' (> 3·5 Å) it is the sandwich form that is stable. Similar results have been obtained for anthracene excimer also (Table IV). Further, for any given value of D (and α) triplet excimer is more stable than the singlet excimer. This result contradicts the experimental data[12]. The possible reasons for this discrepancy is under investigation. In this context, it is interesting to note the observation of Schweitzer *et al*[18], that the wavefunction in a singlet excited state is more diffuse than the wavefunction for the corresponding triplet state, which is rather contracted towards the nuclei. This result together with the increasing feeling that one must use different parameters for singlet and triplet states[19] may be a source for seeking an explanation for the observed differences in stability (and possibly structure) of singlet and triplet excimer. Another important factor, which has great influence on the calculations, is the choice of orbital exponent. In this and earlier works, a constant value of Z = 3·18 has been employed for all distances. Different sets of values for different distances could be expected to yield interesting results.

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APPENDIX

For the evaluation configuration interaction (CI) matrix elements, we follow the method of Pariser[14]. In the case of naphthalene excimer, the CI matrix is a 17 × 17 determinant, while for anthracene excimer, it is 37 × 37 (because we have a pair of degenerate subjacent orbitals in anthracene). The evaluation of the various inter- and intra-molecular integrals are as follows :

Integral	Over monomer	Intermolecular
Overlap	Parr and Crawford[15]	Parr and Crawford[15]
Coulomb/ exchange (with ZDO approximation)	Mataga and Nishimoto[16]	Parr's multipole[17] expansion
core H_{ij}	HMO $E_i \ \delta_{ij}$	$-10{\cdot}0 \ S_{ij}$

Rest of the calculation/procedure is identical with our earlier work[10].

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1. Birks, J. B., *Rep. Prog. Phys.*, 1975, 38, 903.
2. Gordon, M. and Ware, W. R., *The Exciplex*, Academic Press, N.Y., 1975.
3. Forster, Th., *Angew. Chem. Int. Edn. Engl.*, 1969, 8, 333.
4. Murrell, J. N. and Tanaka, J., *Mol. Phys.*, 1964, 4, 363.
5. Konijengerg, E., *Thesis*, Free University, Amsterdam, Holland, 1963.
6. (*a*) Azumi, T. and McGlynn, S. P., *J. Chem. Phys.*, 1964, 41, 3131.
   (*b*) Azumi, T., Armstrong, A. T. and McGlynn, S. P., *Ibid.*, 1964, 41, 3839.
   (*c*) — and McGlynn, S. P., *Ibid.*, 1965, 42, 1675.
7. — and Azumi, H., *Bull. Chem. Soc. Japan*, 1966, 39, 2317.
8. Chandra, A. K. and Lim, E. C., *J. Chem. Phys.*, 1968, 48, 2589 ; *Ibid.*, 1968, 49, 5066.
9. Berson, J. A. and Salem, L., *J. Am. Chem. Soc.*, 1972, 94, 8917.
10. Sudhindra, B. S. and Chandra, A. K., *Mol. Phys.*, 1975, 30, 319.
11. Ferguson, J., Morita, M. and Puza, M., *Chem. Phys. Lett.*, 1976, 42, 288, and references combined therein.
12. (*a*) Takemura, T., Aikawa, M., Baba, H. and Shindo, Y., *J. Am. Chem. Soc.*, 1976, 98, 2205.
    (*b*) Aikawa, M., Takemura, T. and Baba, H., *Bull. Chem. Soc. Japan*, 1976, 49, 431.
13. (*a*) Subudhi, P. C., Kanamara, N. and Lim, E. C., *Chem. Phys. Lett.*, 1975, 32, 503.
    (*b*) — and Lim, E. C., *Ibid.*, 1976, 44, 479.
14. Pariser, R., *J. Chem. Phys.*, 1956, 24, 250.
15. Parr, R. G. and Crawford, B. L. Jr., *Ibid.*, 1948, 16, 1049.
16. Mataga, N. and Nishimoto, K., *Z. Physik. Chem.* (Frankfurt), 1957, 12, 335 ; *Ibid.*, 13, 140.
17. Parr, R. G., *J. Chem. Phys.*, 1960, 33, 1184.
18. Schweitzer, D., Clopa, J. P., Behnke, J., Hausser, K. J., Heneel, H. and Staab, H. A., *Chem. Phys.*, 1975, 11, 373, and references therein.
19. (*a*) Zerner, M. C., *Theoret. Chem. Acta*, (*Berl.*), 1976, 42, 223.
    (*b*) Gondo, Y. and Kanda, K., *Bull. Chem. Soc. Japan*, 1972, 45, 1612.

CHANGES IN SOLUBLE PROTEINS AND ISOENZYMES IN DEVELOPING SORGHUM GRAINS

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ABSTRACT

Electrophoretic patterns of soluble proteins and isoenzymes of peroxidase and esterase showed qualitative and quantitative differences during grain development. The increase in the intensity of protein bands with low electrophoretic mobility at mature stage suggests synthesis of proteins with higher molecular weight.

INTRODUCTION

SOLUBLE proteins are the physiologically active fractions which constitute major bulk of enzymes involved in plant metabolism. Several workers have observed marked qualitative and quantitative changes in soluble proteins and isoenzyme patterns during grain development of wheat[1,2], maize[3] and barley[4]. The specificity of enzyme pattern implies a role of specific enzymes and isoenzymes in development and differentiation[5]. In the present investigation soluble protein and isoenzyme changes in sorghum have been studied during grain development.

MATERIAL AND METHOD

Sorghum (variety CSH-2) was grown at I.A.R.I. farm. The cobs were harvested at 10, 17, 24 and 31 days (mature) after ear emergence. At 10 days, seeds were taken along with husk.

For proteins and esterase extraction, fresh seeds were hand ground at 0–4° in a chilled pestle-mortar with 50 mM Tris-Cl buffer [(pH 7·6) containing 5 mM 2-mercaptoethanol]. For the extraction of peroxidase 2-mercaptoethanol was omitted. The extracted material was centrifuged at 10,000 × g for 20 min. An aliquot of the supernatant (200–250 μg protein) was layered on polyacrylamide gel (10%) and electrophoresis was done using cationic system[6] for soluble proteins. Isoenzymes were separated by anionic system[7,8] using 7% gel. The gels were stained for protein in 0·1% amido black and destained by diffusion in 7% acetic acid.

Detection of esterase isoenzymes.—Gels were incubated in 50 mM phosphate buffer (pH 6·0) containing 1 ml of 1% α-naphthyl acetate in 60% acetone and 25 mg fast blue RR at room temperature for 10–30 min.

Detection of peroxidase isoenzymes.—Gels were first incubated for 30 min in the reaction mixture containing 0·5% O-dianisidine-HCl, 1 ml; 0·6 M NaOAc buffer, 3 ml; and H_2O, 26 ml. Then the gels were incubated in 0·1 MH_2O_2 until the visible bands developed. The gels were preserved in 7% HOAc.

A control gel for each enzyme was incubated in the mixture without substrate. In such controls no bands were observed. At least two independent extractions were made. The R_f of each band with respect to front formed by the tracking dye was calculated. Densitometer tracings of the gels were obtained on a Joyce-Loebl chromoscan.

RESULTS AND DISCUSSION

Soluble proteins

The soluble protein pattern from the seeds at different stages of grain development is shown in Fig. 1. The number of bands decreased with maturity. Band at R_f 0·04 was present at 17 days stage, while R_f 0·11 band was present only in mature grains. Bands with R_f 0·09 and 0·22 were present only at 10 days stage.

Bands at R_f 0·13, 0·17, 0·25, 0·46, 0·48, 0·57, 0·63, 0·69, 0·74, 0·80, 0·85, 0·91 and 0·95 were common to all stages of grain development. With the development of the grain, the bands with medium electrophoretic mobility decreased while bands with high electrophoretic mobility showed a slight increase.

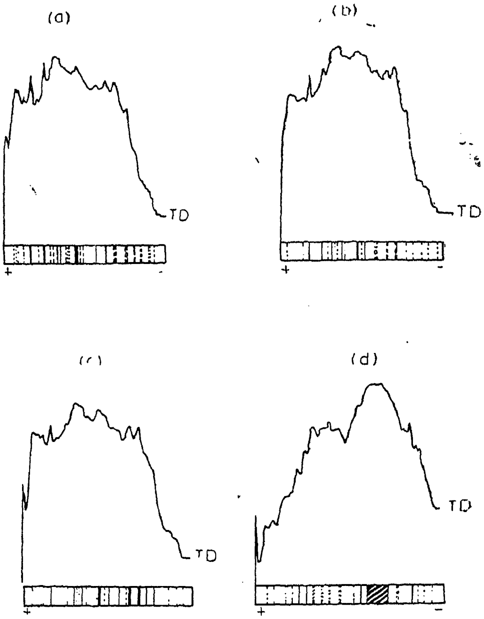

FIG. 1. Soluble protein pattern at (*a*) 10 days, (*b*) 17 days, (*c*) 24 days and (*d*) 31 days.

Increase in the intensity of low electrophoretic mobility bands was observed at mature stage. This may be due to the synthesis of new high molecular weight protein and/or anionic proteins. It is more likely that new proteins of high molecular weight are synthesized during later stages of maturity. Disappearance of bands with medium electrophoretic mobility (R_f 0·31, 0·33, 0·39 and 0·51) during grain development suggests that these proteins represent metabolic proteins rather than storage proteins. Lodha *et al.*[9] also observed a decrease in the number of bands during grain development in normal and opaque-2 maize. Many of the bands at all stages of development were common, thereby suggesting that they are stable proteins and may be involved in storage protein biosynthesis.

Esterase.—The isoenzyme patterns are shown in Fig. 2. The number of esterase isoenzymes were found to be 7 at 10 days stage and 5 at 31 days stage. The bands at R_f 0·00, 0·45, 0·76 and 0·88 were common to all stages, while band at R_f 0·13 and 0·59 present at 10 and 17 days stage disappeared at later stages and instead the band at R_f 0·18 appeared anew during later stages of maturity.

Peroxidase

Electrophoretic patterns of peroxidase from grains are shown in Fig. 2. Five bands were present at 10, 17 and 24 days stage. The intensities of these bands varied considerably. The bands with R_f 0·39 and 0·76 were lighter at 10 and 17 day stage compared to 24 day stage. The bands with R_f 0·12, 0·39 and 0·55, present at earlier stages, disappeared at 31 days, while bands at R_f 0·00 and 0·27 appeared anew. A total of only four bands were present in mature grains. Disappearance of existing bands and appearance of new bands suggest the shifts of specific isoenzymes[10]. The number of peroxidase bands present in sorghum grain are lesser than the number of bands present in maize[9].

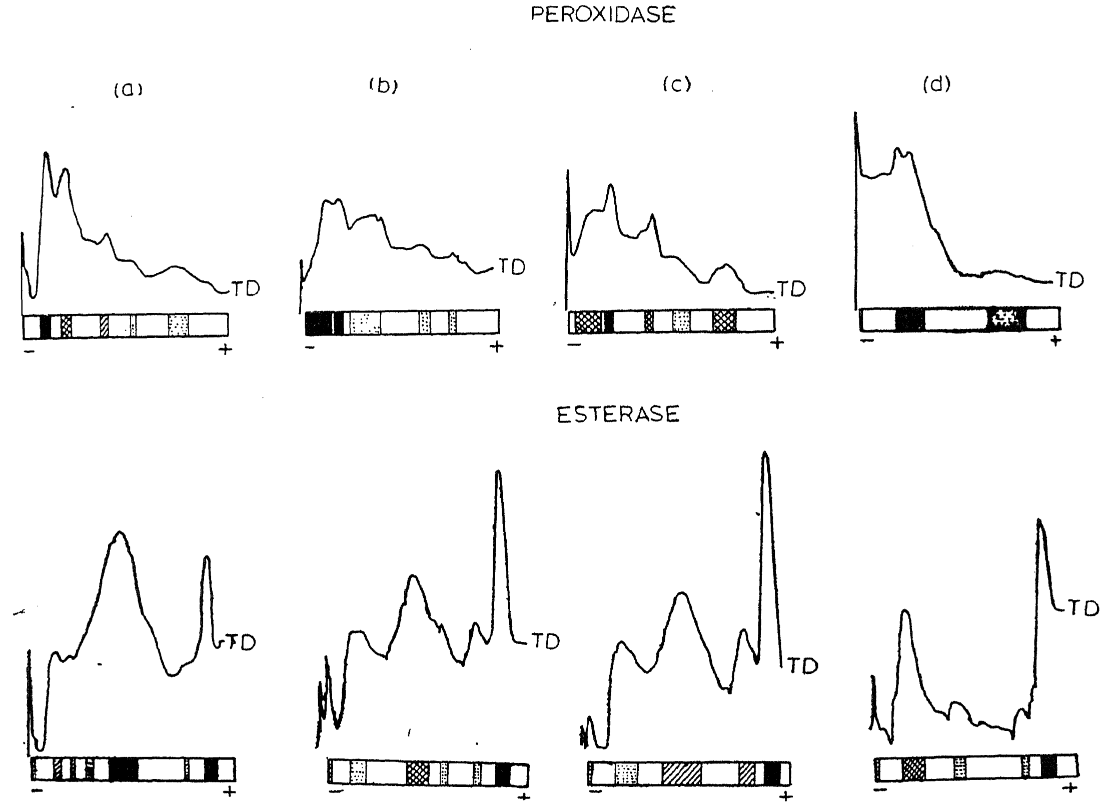

FIG. 2. Peroxidase and esterase isoenzyme pattern at (*a*) 10 days, (*b*) 17 days, (*c*) 24 days and (*d*) 31 days.

The changes observed in peroxidase during grain development may be due to differential gene activity or due to different cellular environment. The exact physiological role of peroxidase in plant is not known. One of the suggested roles of these enzymes include oxidation of certain toxic phenols and amines which may be deleterious to the plants under certain circumstances. Sorghum grains contain tannins which bind with proteins. The effect of peroxidase on tannin contents is not clear but it is likely that these enzymes can change the properties of phenolic compounds by oxidation, which might affect protein-tannin complexes.

1. Bhatia, C. R. and Nilson, J. P., *Biochem. Genet.*, 1969, 3, 207.
2. Macko, V., Honold, G. R. and Stahmann, M. A., *Phytochemistry*, 1967, 6, 465.
3. Kadam, S. S., Singh, Joginder and Mehta, S. L., *Ibid.*, 1973, 12, 1221.
4. Upadhya, M. D. and Yee, J., *Ibid.*, 1968, 7, 937.
5. Market, C. L. and Moller, F., *Proc. Natl. Acad. Sci.*, USA, 1959, 45, 753.
6. Reisfeld, R. A., Lewis, U. J. and Williams, D. E., *Nature*, 1962, 195, 281.
7. Ornstein, L., *Ann. N.Y. Acad. Sci.*, 1964, 121, 321.
8. Davis, B. J., *Ibid.*, 1964, 121, 404.
9. Lodha, M. L., Mali, P. C., Aggarwal, A. K. and Mehta, S. L., *Phytochemistry*, 1974, 13, 539.
10. Scandalios, J. G., *Ann. Rev. Biochem. Genet.*, 1969, 3, 37.

LETTERS TO THE EDITOR

RKRV FRANCK-CONDON FACTORS AND *r*-CENTROIDS FOR THE BAND SYSTEMS OF BO AND BiCl MOLECULES

A PRECISE knowledge of the potential energy as a function of the internuclear distance for molecules of astrophysical importance is needed to obtain accurate values of Franck-Condon (FC) factors and *r*-centroids. It is established that the RKRV potential is more realistic than the other potentials. The present note, therefore, reports the FC-factors and *r*-centroids computed using RKRV potentials for the ($B^2\Sigma^+ - X^2\Sigma^+$) and ($A^2\Pi - X^2\Sigma^+$) transitions of BO molecule and $A^1\Pi - X^1\Sigma$ transition of BiCl molecule.

The method of computation of these parameters using wavefunctions appropriate to RKRV potential energy curves has been already discussed[1] and the same procedure is followed in the present study. The results thus obtained are tabulated in Table I.

TABLE I

v', v''	FC-factors*	*r*-centroids (Å)	Wavelength (Å)
	BO ($B^2\Sigma^+ - X^2\Sigma^+$)		
0,0	1·941–2	1·242	2331·33
0,1	3·031–1	1·290	2437·10
0,2	2·987–1	1·318	2551·40
0,3	1·841–1	1·356	2675·27
0,4	1·493–1	1·388	2809·90
1,1	1·321–1	1·253	2364·54
1,3	2·732–1	1·328	2588·01
1,4	3·277–1	1·368	2713·81
1,5	2·042–1	1·399	2850·56
2,2	2·807–1	1·266	2398·53
2,5	2·701–1	1·378	2753·38
2,6	2·881–1	1·409	2892·23
2,7	2·051–1	1·423	3043·64
3,4	1·123–1	1·313	2544·25
3,7	2·018–1	1·419	2934·90
3,8	2·133–1	1·435	3088·63
	BO ($A^2\Pi - X^2\Sigma^+$)		
0,0	3·619–2	1·274	4247·9
0,1	1·332–1	1·303	4612·7
0,2	2·255–1	1·331	5040·1
0,4	1·831–1	1·385	6160·2
1,0	1·068–1	1·256	4035·5
1,1	1·881–1	1·282	4363·4
1,2	9·551–2	1·311	4744·0
2,0	1·641–1	1·237	3847·0
2,1	1·077–1	1·265	4143·1
2,3	8·813–2	1·321	4883·4
2,4	1·013–1	1·345	5351·0

v', v''	FC-factors*	*r*-centroids (Å)	Wavelength (Å)
	BiCl ($A^1\Pi - X^1\Sigma^+$)		
0,0	1·044–2	2·563	4594·9
0,1	4·881–2	2·588	4660·5
0,2	1·089–1	2·615	4727·5
0,3	1·513–1	2·641	4796·2
0,4	1·468–1	2·670	4866·5
0,5	1·412–1	2·697	4938·1
1,0	3·704–2	2·544	4549·8
1,1	1·021–1	2·572	4614·2
1,2	1·001–1	2·598	4679·9
1,3	3·138–2	2·625	4747·5
1,4	1·335–2	2·650	4815·6
1,5	3·218–2	2·670	4886·9
2,0	6·897–2	2·526	4506·9
2,1	9·667–2	2·555	4569·7
2,2	2·132–2	2·580	4634·3
2,3	2·897–2	2·604	4700·1
2,4	3·199–2	2·631	4767·4
2,5	3·012–2	2·660	4837·3
3,0	9·399–2	2·513	4465·6
3,1	5·137–2	2·537	4527·5
3,2	1·444–2	2·562	4590·7
3,3	3·239–2	2·588	4655·1
3,4	4·015–2	2·616	4721·7
3,5	4·815–2	2·646	4789·8
4,0	1·049–1	2·501	4425·9
4,1	1·452–2	2·524	4487·5
4,2	1·889–2	2·548	4548·9
4,3	1·979–2	2·573	4613·0
4,4	2·815–2	2·599	4677·7
5,0	9·499–2	2·493	4388·0
5,1	7·498–2	2·511	4447·7
5,2	6·185–2	2·535	4508·7
5,3	4·989–2	2·558	4572·4
5,4	2·556–2	2·582	4635·4

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* The negative numerical in each FC-factor entry is the power of 10 by which it is multiplied.

A comparison of the FC-factors and *r*-centroids calculated from RKRV potentials for most of the bands reported here with those calculated[2,3] using Morse potentials shows that the latter results always differ by about 20% for the strong bands and about 45% for the weak bands. This deviation in the results clearly shows that the Morse FC-factors and *r*-centroids cannot be used for further calculations.

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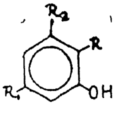
1. Mummigatti, V. M. and Jyoti, B. G., *Curr. Sci.*, 1977, 46 (8), 256.
2. Walvekar, A. P., *Ph.D. Thesis*, Karn. Univ., 1961.
3. Singh, J., Nair, K. P. R. and Rai, D. K., *Indian J. Pure-Appl. Phys.*, 1970, **8**, 614.

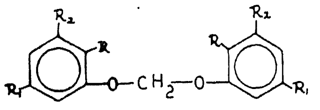
METHYLENATION OF CHELATED HYDROXYLS

Methylenation, regarded as a natural process, has also been reported in the plant polyphenolics[1-3]. Methylenation of carbonyl derivatives of resorcinol[4] and hydroquinone[5] have been reported to yield bimolecular compounds linked by a methylenedioxy group in the reactive 4 and 5 positions respectively. It was reported[4] that the chelated hydroxyl groups do not enter into methylenation. This prompted us to record our observations in this field.

Methylenation of 2-hydroxy-4-methoxy- and 2-hydroxy-4, 6-dimethoxy-acetophenones with methylene iodide in the presence of potassium carbonate and acetone, under reflux for 60 hours, yielded products which have been assigned structures as R—O—CH_2—R on the basis of the following observations.



(1)



(2)

The presence of a methylenedioxy group in these compounds is indicated by the positive gallic acid-sulphuric acid test[6] as well as by a characteristic band at 1040 cm^{-1} in the IR spectra[6]. The absence of normal OH absorption in the IR spectra of the methylenated products indicated that the phenolic OH is involved in methylenation. This is further supported that in the IR spectra of the compounds, the band at 1630 cm^{-1} for the chelated carbonyl disappeared and the band at 1700 cm^{-1} appeared due to the presence of free carbonyl group. The compounds obtained after methylenation gave the satisfactory elemental analysis.

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1. Gopinath, K. W., Kidwai, A. R. and Prakash, L., *Tetrahedron*, 1961, 16, 201.
2. Birch, A. J., Moore, B., Mukerjee, S. K. and Bevan, C. W. L., *Tetrahedron Letters*, 1962, **15**, 673.
3. Khanna, R. N. and Seshadri, T. R., *Tetrahedron*, 1965, **19**, 219.
4. Grover, S. K., Jain, A. C. and Seshadri, T. R., *Ibid.*, 1964, **20**, 555.
5. Gandhi, P. and Tiwari, R. D., *Curr. Sci.* 1976, **45**, 18.
6. Suginome, H., *Tetrahedron Letters*, 1960, **19**, 16.

FATTY ACIDS OF RADIATION-RESISTANT *MICROCOCCUS RADIOPHILUS*

During the past two decades, *Micrococcus* sp. isolated from various sources including food[2,11], animal faeces[10] and radioactive waste water[13], have been found to possess certain morphological and biochemical characteristics which markedly differ from those of micrococci which have been described in Bergey's Manual. In 1973, Lewis[12] had suggested a modification of the Key in Bergey's Manual used for differentiating micrococci, to enable suitable placement for these radio-resistant species. Recently[14], electron microscopic studies have revealed distinct differences in the ultrastructure of these radio-resistant micrococci as compared with other species of the genus *Micrococcus*, and it was recommended that these organisms be placed in a new separate genus.

In recent years, several workers[1,8] have shown close similarities in the fatty acid complements of bacteria belonging to the same genus. It was therefore of interest to examine whether the fatty acids of the radio-resistant *Micrococcus radiophilus*, isolated in our laboratory from irradiated Bombay duck (*Harpodon nehereus*)[11] resemble those of other micrococci.

M. radiophilus, an orange pigmented Gram + ve aerobe was cultured in TGYM media containing 5 g tryptone, 1 g glucose, 3 g yeast extract and 50 mg DL-methionine per litre of tap water. Stationary phase cultures harvested after 24 h incubation on a reciprocatory shaker, were used in these investigations. Cells were centrifuged and washed twice with 0·1 M phosphate buffer (pH 7·0).

TABLE I

Fatty acid composition of M. radiophilus

Fatty acid	14:0[a] br[b]	15:0 br	14:2	16:0 br	16:0	16:1	17:0	16:2	18:0	18:1	18:2
Relative percentage[c]	6·87	8·50	1·87	12·96	3·75	23·50	8·50	23·50	6·25	1·33	3·34

a. No. of carbon atoms in acid : No. of double bonds.
b. *br* = Branched chain.
c. each value is the average of three independent experiments.

TABLE II

Name of microorganism (Gram stain)		Per cent fatty acid			Reference
		n-saturated	*n*-unsaturated	branched	
Micrococcus lysodeikticus	(+)	5	..	93	3
Micrococcus lysodeikticus	(+)	7	2	87	6
Micrococcus agilis	(+)	19	..	79	6
Micrococcus radiodurans	(±)	18	78	..	6
Micrococcus radiodurans	(±)	25	75	..	9
Micrococcus denitrificans	(−)	21	78	..	6
Micrococcus cerificans	(−)	27	67	..	6
Micrococcus halodenitrificans	(−)	21	76	..	8
*Micrococcus radiophilus**	(+)	19	53	28	

* Data taken from Table 1.

Total fatty acids were extracted from wet cells by saponification according to the method of Fulco *et al.*[4] Fatty acids were converted into methyl esters using the procedure of Hancock and Meadow[7], and analysed in a gas chromatograph (Bhabha Atomic Research Centre model) equipped with an hydrogen flame ionization detector. Separation of methyl esters was accomplished in a coil-shaped stainless steel column (6 ft long, O.D. 0·25 in.) packed with 20% DEGS on Chromosorb P and maintained at 187° C. N_2 was used as the carrier gas. The injection port temperature was 227° C. Peaks were identified on the basis of their retention time, using standard fatty acid methyl esters. Quantitative assessment was made by the triangulation method. Absence of hydroxy fatty acids was judged by adsorption TLC[15].

The total fatty acid composition of *M. radiophilus* has been presented in Table I. As can be seen from the table, this organism comprises about 70% normal straight chain acids and about 30% branched chain acids. In this respect *M. radiophilus* varies significantlyf rom the radio-resistant Gram variable *Micrococcus radiodurans* which is devoid of branched chain acids as well as from other Gram + ve micrococci like *Micrococcus lysodeikticus* and *Micrococcus agilis* which possess more than 80% branched chain acids, and Gram − ve micrococci like *Micrococcus denitrificans*, *Micrococcus cerificans* and *Micrococcus halo-*

denitrificans which contain a major proportion of unsaturated fatty acids (Table II). One of the striking features of the fatty acid complement of *M. radiophilus* is the large quantity of even numbered poly-unsaturated acids. The occurrence of polyunsaturated fatty acids in bacteria is rare—these poly-unsaturated acids have so far been reported mainly in *Bacillus* sp.[5]

The outstanding differences in the fatty acid spectrum of *M. radiophilus*, as compared with those of other micrococci, support our earlier suggestions[12, 14], that the placement of this bacterium in the genus *Micrococcus* should be reconsidered.

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1. Abel, K., DeSchmertzing, H. and Peterson, J. I., *J. Bacteriol.*, 1963, **85**, 1039.
2. Anderson, A. W., Nordan, H. C., Cain, R. F., Parrish, G. and Duggan, D., *Food Technology*, 1956, **10**, 575.
3. Cho, K. Y. and Salton, M. R. J., *Biochim. Biophys. Acta*, 1966, **116**, 73.
4. Fulco, A. J., Levy, R. and Bloch, K., *J. Biol. Chem.*, 1963, **239**, 998.
5. —, *Ibid.*, 1969, **244**, 889.
6. Girard, A. E., *Can. J. Microbiol.*, 1971, **17**, 1503.
7. Hancock, I. C. and Meadow, P. M., *Biochim. Biophys. Acta*, 1969, **187**, 366.
8. Kates, M., *Adv. Lipid Res.*, 1964, **2**, 17.
9. Knivett, V. A., Cullen, J. and Jackson, M. J., *Biochem. J.*, 1965, **96**, 2c.
10. Kobatake, M., Tanabe, S. and Hasegawa, S., *C.R. Soc. Biol. (Paris)*, 1973, **167**, 1506.
11. Lewis, N. F., *J. Gen. Microbiol.*, 1971, 66, 29.
12. Lewis, N. F., *Curr. Sci.*, 1973, **42**, 504.
13. Matsuyama, A., In *Radiation Preservation of Food*, IAEA, Vienna. 1973, pp. 261,
14. Sleytr, U. B., Silva, M. T., Kocur, M. and Lewis, N. F., *Arch. Microbiol.*, 1976, **107**, 313.
15. Yano, I., Furukawa, Y. and Kusunose, M., *Biochim. Biophys. Acta*, 1969, **187**, 166.

DIFFERENTIAL PRECIPITATION OF GLYCOSAMINOGLYCANS BY ZINC AT pH 7·0

While studying zinc binding by each of the seven different glycosaminoglycans (GAGs)[1], we observed that when slightly acidic solutions containing the sodium salt of a GAG and $^{65}ZnCl_2$ were adjusted to pH 7·0, the amounts of ^{65}Zn precipitated were relatively constant, leaving 41·4 to 49·0% of the total ^{65}Zn in the supernates (Table I). However, in contrast to ^{65}Zn, the amounts of GAGs remaining in the supernates ranged from 3·8 to 90·2% of the total GAGs.

Table I

Percentages of GAGs and ^{65}Zn found in pH 7·0 supernates

Samples	GAGs (%)	^{65}Zn (%)
Chondroitin-4-sulfate	68·1	43·4
Chondroitin-6-sulfate	48·5	42·8
Dermatan sulfate	64·3	42·9
Heparan sulfate	3·8	41·4
Heparin	7·8	47·0
Hyaluronic acid	73·1	49·0
Keratan sulfate	90·2	45·5

The values reported in Table I were obtained from samples containing 600 μg of a specified, reference standard GAG (gift of Dr. J. A. Cifonelli, University of Chicago) and 10 μmoles of $^{65}ZnCl_2$. Each sample was adjusted to pH 7·0 by the addition of dilute NaOH and made to 320 μl with water. After setting aside at room temperature for 15 min and centrifuging, the supernates were measured for hexuronic acid content by the *m*-hydroxydiphenyl method[2] and galactose by the anthrone method[3]. The radioactivity of ^{65}Zn was measured with the Packard Gamma Scintillation Spectrometer. Aliquots of a working solution of $^{65}ZnCl_2$ were used as reference standard. The working solution of $^{65}ZnCl_2$ was prepared by adding an aliquot of $^{65}ZnCl_2$ (1–10 Ci/g zinc in 0·5 N HCl, purchased from New England Nuclear, Boston, Massachusetts 02118) to an aqueous solution of $ZnCl_2$, adjusting to pH 4·0 with 0·01 N NaOH and diluting to 1·000 M zinc chloride, inclusive of $^{65}ZnCl_2$. Each GAG was dissolved in glass-distilled deionized water, adjusted to pH 4·0 with NaOH, and diluted to a concentration of 2·0 mg GAG/ml.

Appropriate precautions were taken with glassware and equipment to prevent contamination with extraneous zinc. Syringes (Hamilton Co., Reno, Nevada) were washed with 0·02 N HCl and rinsed thoroughly with water and with the solution to be measured just before use.

Because zinc is an ampholyte (having both acidic and basic functions in presence of strong bases and strong acids respectively[4]), its solubility in acidic and basic solutions was ascertained. At pH 4·0, 10 μ moles of $^{65}ZnCl_2$ produced no precipitate in 1·0 ml of 0·1 M NaCl solution. At pH 7·0, 4·0μ moles of ^{65}Zn were found in the supernate and this value was not affected by NaCl concentrations of 5×10^{-6} to 10^{-1} M. Minimum solubility of ^{65}Zn in 0·1 M NaCl was observed at pH 11·0; less than 0·1 μmole of ^{65}Zn/ml was found in the supernate. In the absence of zinc, but otherwise identical conditions, GAGs did not form any precipitate.

The differential precipitation of GAGs was observed at pH 7·0, where 51·0 to 58·6% of the total zinc had sedimented. Thus, one might expect that there was some coprecipitation of GAG with zinc. However, the wide range in GAG solubility (3·8 to 90·2%) indicates that coprecipitation was not purely mechanical. Rather, it appears that GAG solubility in neutral zinc solution is roughly related to GAG composition and structure[5] (Table I): Keratan sulfate, the least precipitable GAG, has practically no hexuronic acid (1·9%) by weight, given with the sample), unlike the other GAGs. The most precipitable GAGs, heparan sulfate and heparin, are the only GAGs having sulfamino groups; they also have ester sulfate groups and hexuronic acids. Hyaluronic acid has hexuronic acids but no ester sulfates; only 27% of the total was precipitated. Chondroitin-4-sulfate, chondroitin-6-sulfate, and dermatan sulfate have hexuronic acids and ester sulfates; intermediate amounts of these GAGs were precipitated. The GAG solubility, in addition, seems partly related to zinc-binding capacity[1]: Of the GAGs, keratan sulfate binds lowest amounts of zinc; heparin binds highest amounts (heparan sulfate, slightly less) of zinc; and chondroitin-4-sulfate, chondroitin-6-sulfate, and dermatan sulfate bind intermediate amounts of zinc. Hyaluronic acid also binds highest amounts of zinc, but unlike heparin, its zinc-binding capacity decreases greatly with decreasing pH. Zinc-binding capacity of dermatan sulfate, while less than that of chondroitin-6-sulfate, approximates that of chondroitin-4-sulfate. (This trend also appeared in Table I.)

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1. Sato, C. S. and Gyorkey, F., *J. Biochem.*, 1976, **80**, 111.
2. Blumenkrantz, N. and Asboe-Hansen, G., *Anal. Biochem.*, 1973, **54**, 484.
3. Spiro, R. G., *Meth. Enzymol.*, 1966, **8**, 3.
4. Partington, J. R., *A Text-Book of Inorganic Chemistry*, 6th Edition, MacMillan, London, 1950, p. 780.
5. Balazs, E. A., *Chemistry and Molecular Biology of the Intercellular Matrix*, Vol. 2, Academic Press, New York, 1970, p. 705.

EFFECT OF CHLORMADINONE ACETATE ON THE WEIGHT AND CHOLESTEROL CONTENT OF THE RAT ADRENAL

USE of the potent synthetic progestin chlormadinone acetate (6-chloro-6-, 17α-acetoxyprogesterone) has become widespread in clinical contraception[6]. Recently chlormadinone acetate has also been shown to produce functional sterility in the male rat[2,7]. While examining the antifertility effects of low doses of chlormadinone acetate in the male rat it was considered worthwhile to investigate if the progestin had any effect on the adrenal.

Wister albino male rats (300–325 g body wt.) of proven fertility from the departmental colony were used for this study. The control and 3 experimental groups each contained 6 animals which were housed individually. Animals in the experimental groups received a daily intramuscular injection of 0·2, 0·5 or 1·0 mg chlormadinone acetate in 0·1 ml olive oil for 40 days. Controls were similarly injected with the vehicle alone. Autopsies were made 24 hr after the last injection. Adrenals were taken out, cleared from adhering tissue and weighed to the nearest mg. The total cholesterol content of the adrenals was quantitatively determined by the method of Zak as described by Hawk *et al*[5]. The results were analysed by "Student's" *t*-test[3].

With increasing doses of chlormadinone acetate, the weight of the rat adrenals correspondingly decline (Table I). However, the total cholesterol content of the adrenals increases with the dose and a statistically highly significant increase ($p < 0.001$) results at the 1·0 mg/day dose.

There is substantial experimental evidence to support the view that the most important precursor of steroidal hormones, whether adrenal[1] or gonadal[4], is cholesterol. Effect of chlormadinone acetate on the adrenals of the rat could be visualised in this light. Thus the progestin may possibly influence synthesis of adrenocorticoids from cholesterol by blocking the enzyme system at one or more steps of the biosynthetic pathway causing accumulation of cholesterol and depletion of steroids, the latter being one of the important possible causes of the decrease in weight of the gland. Due to lack of experimental evidence it is difficult

TABLE I

Effect of chlormadinone acetate for 40 days on the weight and cholesterol content of the adrenal of the male rat 6/group, mean value ± S.E.M.

Group	Total weight (mg)	Total cholesterol (mg/gm tissue)
Vehicle control	14·15±1·09	15·3±1·59
Chlormadinone acetate:		
0·2 mg/day/rat	13·52±1·23	19·3±2·13
0·5 mg/day/rat	10·63±0·45*	31·6±2·57**
1·0 mg/day/rat	10·47±1·01*	37·9±3·27***

Significantly different compared with control: $^{*}p < 0{\cdot}05$; $^{**}p < 0{\cdot}01$; $^{***}p < 0{\cdot}001$.

to surmise whether the inhibitory action of chlormadinone acetate on the rat adrenal is direct or indirect. It may be mentioned that increasing doses of chlormadinone acetate which were employed in this experiment also caused a complimentary dose-dependent decrease in the weight and gonadotropin content of the pituitary along with gonadal function[7]. A similar type of mediation of action of chlormadinone acetate through the hypophyseal-adrenal axis can be conjectured although on a purely hypothetical basis.

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---

1. Dorfman, R. I., *Cancer*, 1957, 10, 741.
2. Dörner, G., Gotz, F. and Mainz, K., *J. Endocr.*, 1972, **52**, 197.
3. Fisher, R. A., *Statistical Methods for Research Workers*, Hafner Publ. Co., N.Y., 1958.
4. Hall, P. F., *The Testis*, Vol. II, Academic Press, N.Y., 1970.
5. Hawk, P. B., Oser, B. L. and Summerson, W. H., *Practical Physiological Chemistry*, McGraw-Hill, N.Y., 1954.
6. Martinez-Manautou, J., Giner-Velasquez, J. and Rudel, H., *Fertil. Steril.*, 1967, **18**, 57.
7. Sharma, M. M., Lal, G and Jacob, D., *J. Reprod. Fert.*, 1976, **48**, 177.

EXPOSURE OF WHITE MICE TO *BACULOVIRUS AMSACTA* OF GROUNDNUT RED-HAIRY CATERPILLAR, *AMSACTA ALBISTRIGA* (WALKER)

RECENT interest in the use of the nuclear polyhedrosis virus (NPV) against the groundnut red-hairy caterpillar, *Amsacta albistriga*[1,2] as a possible microbial agent, necessitates to establish whether this virus is pathogenic and/or toxicogenic to other non-target animals. More evidence is being accumulated on the specificity of insect polyhedrosis viruses and their safety to vertebrates[3]. In India, though the usefulness of NPV of *A. albistriga* has been reported by Jayaraj *et al.*[1,2] there has been no study on its safety against the other non-target animals except the recent study of Narayanan *et al.*[4,5] in which the authors have established the innocuous nature of this virus to common carp, *Cyprinus carpio* and to poultry birds. The present report is the result of a similar study of the possible toxicity and/or pathogenicity of NPV of *A. albistriga* to white mice.

The polyhedral inclusion bodies were processed from dead, final instar larvae of *A. albistriga* by differential centrifugation and the final concentration was characterised as to the number of PIBs/ml. To find out the bacterial contamination of the virus preparation, differential bacterial-plate counts were made. The total number of bacterial cells present was estimated by pour plate technique using Difco Nutrient Agar and the presence or absence of coliforms was detected using Mac-Conkey's agar medium. The number of viable spores present was estimated by pasteurization technique. The acute toxicity/pathogenicity evaluation tests were carried out by following standard methods[6].

Two to four weeks old and randomly mated non-inbred lines of white mice (average weight 26·1 g; range 21·4 to 28·6 g) were used in the study. An oral administration of 0·01 ml of virus suspension was given using a 1 ml tuberculin syringe fitted with 5 cm tifflon sleeve of 0·5 mm dia to each of five male and five female mice. The virus suspension was found to contain 1·9 to 2·5 × 10^5 bacterial cells, 2·1 × 10^3 bacterial spores and no coliforms. The dose fed to each mouse was more than 100 times the average field dose/acre with a conversion ratio of the weight of test animal to weight of man. In addition, ten mice, five in each sex, were used as untreated control, receiving 0·01 ml of sterile distilled water. The animals were maintained in separate cages and standard commercial food material along with sliced carrots and water

TABLE I

Results of tests in which white mice were exposed to the nuclear polyhedrosis virus of Amsacta albistriga

Observations	Treated		Control	
	Male	Female	Male	Female
Initial mean weight of animals (g)	28·6	27·0	27·6	21·4
Final mean weight of animals (g)	30·8	31·8	30·0	26·2
Mean weight gain	2·2	4·8	2·4	4·8 NS
Body mean temperature	37·6° C		37·4° C	
Blood glucose (mg/100 ml)	140·0		160·0	
Haemoglobin (g/100 ml)	8·2		8·4	
Haematocrit value (%)	49·0		45·0	
Serum alkaline phosphate m. moles/100 ml/min.	9·5		7·5	
Organs wet-weight (mg)				
Heart	323		354	
Lungs	550		471	
Liver	1768		2012	
Kidney	772		645	
Spleen	352		413	
Ovaries	238		240	
Testes	400		420	

NS = Non-significant.

were supplied *ad libitum*. The test was terminated after 21 days.

Observations on the general conditions of the test animals, and bio-chemical, necropsy and histopathological studies were made to evaluate the effects of the virus. The mice were examined daily for general appearance and behaviour. Body weight and temperature records were taken at weekly intervals until the test was terminated. At the end of the test period, animals were etherised and blood withdrawn by cardiac puncture. Potassium oxalate was used to prevent coagulation. Blood glucose was estimated according to the methods of Somogyi[7]. Serum alkaline phosphatase enzyme was determined using M/100 disodium phenyl phosphate as the substrate with carbonate-bicarbonate buffer (pH 9·2)[8]. The packed cell volume or the haematocrit value was obtained using the Wintrobe haematocrit tubes and blood haemoglobin was estimated using Sahlis' haemoglobinometer[9]. At the end of the test period, the animals were dissected. Various organs for gross-pathology, and wet-weight of the important organs like heart, liver, lungs, spleen, kidney and gonads were examined and recorded. Histopathological observations were also conducted on the above organs following the standard microtome procedures.

The above tests yielded the following results. No death occurred in any of the virus-fed or water-fed animals during the period of the observation. However, one male animal both in treated and water-fed died on 4th and 3rd day respectively. The reason for the death was ascribed to be due to the severe fighting which had occurred when these male animals were put in groups due to the presence of "Bully". The animals were severely mauled and bitten, especially at the back, tail and leg regions. After that these animals were kept in separate cages. All animals were found healthy and there was no change in their bodily appearance, behaviour and feeding activity. Temperature was found normal during the entire test period in both the groups of animals. Body weight gained in each sex of virus treated mice was essentially similar to those of the control. In addition, no difference due to virus treatment could be detected in blood sugar, haemoglobin content and haematocrit value and serum alkaline phosphatase enzyme activity (Table I). Post-mortem examinations indicated no gross pathology in either group. The average weight of each important organ (Table I) did not vary much. Histological examinations of all the tissues examined showed no evidence of tissue damage and they were similar to healthy animals.

Safety of similar insect viruses has been reported earlier in the case of white mice, guinea pigs[10], various mammals, fishes[3], chicks[11] and human beings[12]. Further, the specificity of *A. albistriga* NPV has also been reported when it was tested against 15 species of lepidopteran insects and found to be non-infective[13]. The result of the present study as well as those previously documented for other vertebrates and invertebrates with different insect inclusion viruses, indicate their safety and they are apparently host specific. Hence our study on *Baculovirus amsacta* in mice further corroborates the high specificity and its safety against white mice and its intended use as a viral insecticide.

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---

1. Jayaraj, S., Sundaramurthy, V. T. and Mahadevan, N. R., *Madras agric. J.*, 1976, **63**, 567.
2. —, —, — and Swamiappan, M., *Ibid.*, 1977, **14**, 265.
3. Ignoffo, C. M., *Bull. Entomol. Soc. Amer.*, 1968, **14**, 265.
4. Narayanan, K., Govindarajan, R., Jayaraj, S., Paulraj, S. and Kutty, M. N., *Madras agric. J.*, 1977, **64** (in press).
5. — Santharam, G., Easwaramoorthy, S. and Jayaraj, S., *J. Invertebr. Pathol.*, 1977, **29** (in press).
6. Ignoffo, C. M., *Proc. N.Y. Acad. Sci.*, 1973, **217**, 1965.
7. Somegyi, N., *J. Biol. Chem.*, 1952, **200**, 145.
8. King, E. J. and Armstrong, A. R., *Canad. Med. Assoc.*, 1934, **31**, 376.
9. Schalm, O. W., Jain, N. C. and Carroll, J., *Veterinary Haematology*, Lea and Febiger, Philadelphia, 1975.
10. Ignoffo, C. M. and Heimpel, A. M., *J. Invertebr. Pathol.*, 1965, **7**, 329.
11. Aizawa, K. A., *Entomophaga*, 1961, **3**, 197.
12. Heimpel, A. M. and Buchanan, L. K., *J. Invertebr. Pathol.*, 1967, **9**, 55.
13. Rajamohan, N. and Jayaraj, S., *Curr. Sci.*, 1975, **44**, 274.

ADULT PIGMENTATION AS AN INDEX OF ENDOCRINE ACTIVITY IN THE CRICKET, *GRYLLUS BIMACULATUS*

THE cricket *G. bimaculatus* exhibits orange-yellow/black colour polymorphism. In laboratory population, the females are normally dark with black pigments but males are light coloured with orange-yellow pigments[1,2]. Further, pigmentation is found to be influenced by the corpora allata hormone. While allatectomy resulted in dark colouration, implantation of corpora allata (CA) led to lighter cuticle[2]. Following observations were made in this laboratory.

Topical application of a synthetic juvenile hormone analogue (JHA) (RO–20–3600, Hoffman-La Roche) to the last instar nymphs 96 hr prior to metamorphosis resulted in lighter coloured adults. These results, being statistically significant, support the view that more of juvenile hormone (or active CA) will favour lighter colouration. Isolated rearing of nymphs retarded nymphal growth and development as noted for *P. guttiventris*[3]. Further, male adults were significantly dark coloured as compared with their counterparts, reared in groups. On the basis of earlier results, if endocrine activity is to be correlated with pigmentation, CA in group reared nymphs might be secreting higher level of hormone at least during sclerotisation. However, this indirect evidence does not agree with Gona's supposition[4] that CA might be hyperactive in isolated crickets.

Injection of 1 and 10 μg of thiotepa into the last instar male nymphs resulted in the appearance of completely light coloured adults. This effect was similar to that following JHA application. Again, taking pigmentation as an index of CA activity, thiotepa appears to activate these glands to secrete hormone if injected in last nymphal instar. Though impairment of neuro-endocrine functioning by chemosterilants is noted for *Ephistia kuhniella*[5,6] and *Periplaneta americana*[7], probable 'allatotropic' effect has not been suggested so far. For both isolated rearing and thiotepa treatment females showed less sensitivity towards change in pigmentation. The reason for this is not clear since gonads were not found to play any role in influencing adult pigmentation.

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1. Fuzeau-Braesch, S., *Bull. biol. Fr. Belg.*, 1960, 94, 526.
2. Roussel, J.-P., *J. Insect Physiol.*, 1966, 12, 1085.
3. Dakshayani, K. and Mathad, S. B., *Experientia*, 1973, 29, 978.
4. Gona, O., *Ibid.*, 1976, 32, 44.
5. Tan, K. H., *Ibid.*, 1974, 30, 1403.
6. —, *Physiol. Entomol.*, 1976, 1, 61.
7. Bhargava, S. and Mathur, R., *Chemoster. News*, 1975, 3, 13.

ON THE APPLICATION OF DYAR'S RULE TO THE LARVAE OF *HENOSEPILACHNA VIGINTIOCTOPUNCTATA* (FABRICIUS)

DYAR[3] (1890), while studying the head-capsule width of 28 species of lepidopterous larvae, found that width was relatively static during a stadium and grew in geometrical progression in successive stages of a larva. This growth ratio is constant for a particular species and by using this ratio it was possible to determine the exact number of instars of species. On the other hand Taylor[8] (1931) and Richards[7] (1949) mentioned that progression is rarely regular. Taylor[8] (1931) also suggested a modification of Dyar's Rule. In the present investigation the applicability of Dyar's Rule and Taylor's modification of it in the larvae of *Henosepilachna vigintioctopunctata* (Fabricius) (Coleoptera : Coccinellidae), which is a serious pest of many vegetable crops in India, was tested.

Materials and Methods.—Field collected adults were kept in jars and potato leaves were provided for egg laying. The first instar larvae (0–12 h old) hatching from these eggs were reared individually on potato leaves in plastic petri dishes (10 cm in dia.) at 27 ± 1° C with 60 ± 5% r.h. and photoperiod of 12 h photophase and 12 hr scotophase was also maintained. Head-capsules of each larva were collected and stored in homeopathic vials. The width of the head-capsule was measured across the greatest width of the head, *i.e.*, at the base of mandibles with a stereomicroscope fitted with an ocular micrometer. Modification suggested by Taylor[8] (1931) was also applied. In addition to these, the data were also subjected to regression analysis, *viz.*, linear, exponential, modified exponential and logistic to find out the better fit.

Results and Discussion.—Perusal of Table I reveals that the size of the head-capsule varied greatly but there was no overlapping of sizes between instars. Size of one instar, therefore, cannot be confused with that of another instar. However, the sizes of head-capsules of the two sexes of a particular instar did not differ significantly. When log values of the mean head-capsule width were plotted a linear relationship was not obtained indicating that the head-capsule width

TABLE I

Observed and calculated head width of different instars of H. vigintioctopunctata

Instar	Observed head width (mm)			Calculated head width*
	Range	Mean	Growth ratio	
I	0·369–0·469	0·420	1·44	0·452
II	0·530–0·692	0·603	1·47	0·613
III	0·803–0·953	0·887	1·15	0·782
IV	0·968–1·107	1·018	..	1·180

* Average obtained by applying Taylor's modification.

TABLE II

Analysis of variance of observed head width of H. vigintioctopunctata

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Replications	87	0·126	0·001	..
Treatments	3	19·312	6·437	7152·22**
Linear	1	18·965	18·965	21072·22**
Quadratic	1	0·062	0·062	68·89**
Cubic	1	0·282	0·282	3·1333**
Error	261	0·242	0·0009	
Total	351	19·680		

** Significant at 1% level.

TABLE III

Observed and calculated values for linear, exponential, modified exponential and logistic curves

Observed value of Y	Estimated values of Y							
	Linear regression	Deviation from linear	Exponential	Deviation from linear	Modified exponential	Deviation from linear	Logistic	Deivation from linear
0·4202	0·4206	0·0004	0·4384	0·0182	0·4089	0·0113	0·4186	0·0016
0·6033	0·6282	0·0249	0·5941	0·0092	0·6402	0·0369	0·6018	0·0015
0·8869	0·8358	0·0511	0·8051	0·0818	0·8473	0·0396	0·8409	0·0460
1·0177	1·0434	0·0257	1·0910	0·0733	1·0324	0·0117	1·0491	0·0314
Deviation from regression	0·0039		0·0125		0·0032		0·0031	
$1 - R^2$	0·0177		0·0569		0·1492		0·1416	
R^2	98·22%		94·31%		98·51%		98·58%	

does not increase in geometrical progression. Dyar's Rule was, therefore, not applicable in this case. This rule was also found inapplicable in the female larvae of *Porthetria dispar* Linnaeus (Keler[5], 1933), *Heliothis obsoleta* (Fabricius) (Gains and Cambell[4], 1935), *Spodoptera littoralis* (Boisduval) (Prasad[6], 1973; Bhat[1], 1974) and *Diacrisia obliqua* Walker, (Deshmukh[2], 1976). Growth ratios calculated by diving each mean by the mean preceding it indicated that head-capsules do not grow in the same proportion from instar to instar as the ratio of head width between III : IV instars was quite low as compared to others. Modifications suggested by Taylor[8] (1931) did not provide a perfect fit. However, the data were more close to Taylor's modification than original Dyar's hypothesis.

To know the actual relationship, the data were statistically analysed in a randomized block design considering instars as treatments and number of head-capsules observed in each instar as replications. The treatment sum of squares was partitioned into linear, quadratic and cubic to find out the greatest contribution of a particular function. From Table II, linear seems to be a good fit, as the variation explained by fitting additional terms are relatively very small. Estimation of values for linear, exponential, modified exponential and logistic (Table III) further revealed that head-capsule width does not increase in geometrical progression as suggested by Dyar because exponential was not found giving closest fit in any instar of this insect. Performance of linear is better than exponential. Best ones are modified exponential and logistic. Seeing the ease of computation and non-significant gain by fitting the modifiel exponential and logistic functions, linear function may be regarded as satisfactory fit.

The authors are grateful to Dr. M. C. Saxena, Director, Experiment Station, for providing necessary facilities and Dr. V. P. Manglik, Statistician, for helping us in statistical analysis.

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---

1. Bhat, N. S., *M.Sc. Thesis*, submitted to G. B. Pant University of Agri. and Tech., Pantnagar, 1974.
2. Deshmukh, P. D., *M.Sc. Thesis*, submitted to G. B. Pant University of Agri. and Tech., Pantnagar, 1976.
3. Dyar, H. G., *Psyche*, 1890, 5, 420.
4. Gains, J. C. and Cambell, F. L., *Ann. ent. Soc. Amer.*, 1935, 28, 445.
5. Keler, S., *Polak. Pisoma. Ent.*, 1933, 12, 173.
6. Prasad, J., *M.Sc. Thesis*, submitted to G. B. Pant University of Agri. and Tech., Pantnagar, 1973.
7. Richards, O. W., *Proc. R. Ent. Soc.*, London, 1949, 24 A, 8.
8. Taylor, R. L., *Ann. ent. Soc. Amer.*, 1931, 24, 451.

NEW RECORD OF PREDATORY BEETLES ON *CHILO PARTELLUS* (SWINHOE)

THE maize and *Jowar* borer, *Chilo partellus* (Swinhoe) (Pyralidae : Lepidoptera) is the most destructive pest of maize *Zea mays* Linn. and sorghum *Sorghum bicolor* Moench. in India. While studying the population dynamics of this insect at Ludhiana during 1975–76, four species of beetles including a carabid namely, *Chlaenius, hemifer* Chaudoir, and three coccinellids, *viz.*, *Brumus suturalis* Fabr., *Coccinella septumpunctata* Linn. and *Menochillus sexmaculata* (Fabr.) were recorded preying upon larval and pupal stages of the insect. Black, elongated larvae of *C. hemifer* were found feeding on the larvae and pupae in the tunnels of maize and sorpgum plants. The brownish black beetles have two prominent brownish spots on elytra connected by semi-circular line.

The adults of *B. suturalis, C. septumpunctata*, and *M. sexmaculata* were found feeding on the larvae in the whorl of the maize and sorghum plants. Earlier *C. undecimpunctata* and *M. sexmaculata* have been reported as predators of larvae of *C. partellus* (Jotwani and Verma[6], 1969 ; Jotwani *et al.*[1], 1972). Rathore[3] (1969) reported *Gonocephalum depressum* Fabr. a tenebrionid beetle breying upon the adults of *C. partellus*. The record of three species of beetles, *viz.*, *C. hemifer, C. septumpunctata*, and *B. suturalis* as predators of maize and *jowar* borer larvae and pupae is the first report from India.

The authors thank the Directors, British Museum, London and the Zoological Survey of India, Calcutta, for the identification of beetles.

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Punjab Agricultural University, G. S. SANDHU.
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---

1. Jotwani, M. G., Anand, M. and Lal, R., *Indian J. Ent.*, 1972, 34, 70.
2. — and Verma, K. K., *Ibid.*, 1969, 31, 84.
3. Rathore, Y. S., *Ibid.*, 1969, 31, 287.

ACHLYA CAROLINIANA COKER— A NEW RECORD FROM INDIA

DURING the course of an investigation on fungi associated with fish diseases, *Achlya caroliniana* Coker was isolated from the infected *Channa punctatus* fishes captured from Ramgarh Tal, Gorakhpur, during January 1975. Pure cultures of the fungus were obtained by standard methods and the description corresponded to the bacteria free cultures growing on hemp seed halves in sterile water at 22–25° C. The identity of the isolate was confirmed with the help of monograph of *Achlya by* Johnson[1] (1956).

The cultures have been deposited in Mycological Laboratory of St. Andrew's College, Gorakhpur, at No. F/R-30.

A perusal of the literature had revealed that it is the first report of this fungus from India.

Authors are thankful to Dr. Y. B. Singh, Principal, for providing facilities and to Prof. K. S. Bhargava, Department of Botany, Gorakhpur University, for his valuable suggestions and guidance.

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St. Andrew's College, G. C. SRIVASTAVA.
Gorakhpur, *July* 12, 1976.

---

1. Johnson, T. W., Jr., *The Genus Achlya—Morphology and Taxonomy*, University of Michigan Press, Ann Arbor, Michigan, 1956.

ON TERMINAL SCLEREIDS IN THE COTYLEDONARY LEAVES OF *MEMECYLON UMBELLATUM* BURM. f. (MELASTOMATACEAE)

ON the basis of the published data[1-3], one can conclude that terminal foliar sclereids of monomorphic and polymorphic groupings *sensu* Rao and Bhupal[4] are typical of the genus *Memecylon* as a whole. This generic feature led us to examine the cotyledonary photosynthetic leaves of the seedlings of *M. talboteanum* Brandis and *M. umbellatum* Burm. f. collected in the vicinity of Coondapur.

Karnataka State, India. The present investigation has revealed that terminal filiform sclereids are absent in *M. talboteanum*.

This note presents, in a detailed manner, the terminal sclereids observed in the cotyledonary leaves. The cleared cotyledonary photosynthetic leaves of *M. umbellatum* show filiform sclereids of limited length. They have distinct terminal or sub-terminal relationship with the vein-endings. They vary from each other in length and mode of branching. A few of them show distinct forking and incurved branches of unequal length. They are present in singles or pairs and absent in the vicinity of main veins. They are not so densely distributed as reported in the leaves of this taxa[1]. In transections they are found traversing the mesophyll especially in the spongy region.

We are thankful to Dr. R. Sundar Raghavan, Regional Botanist, Botanical Survey of India, Western Circle, Poona, for the gift of the materials used in this study.

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---

1. Rao, T. A., *Phytomorphology*, 1957, 7, 306.
2. — and Bhupal, O. P., *Proc. Indian Acad. Sci.*, 1974, 80, 291.
3. — and Dakshni, K. M. M., *Ibid.*, 1963, **58 B**, 28.
4. — and Bhupal, O. P., *Ibid.*, 1973, **77 B**, 41.

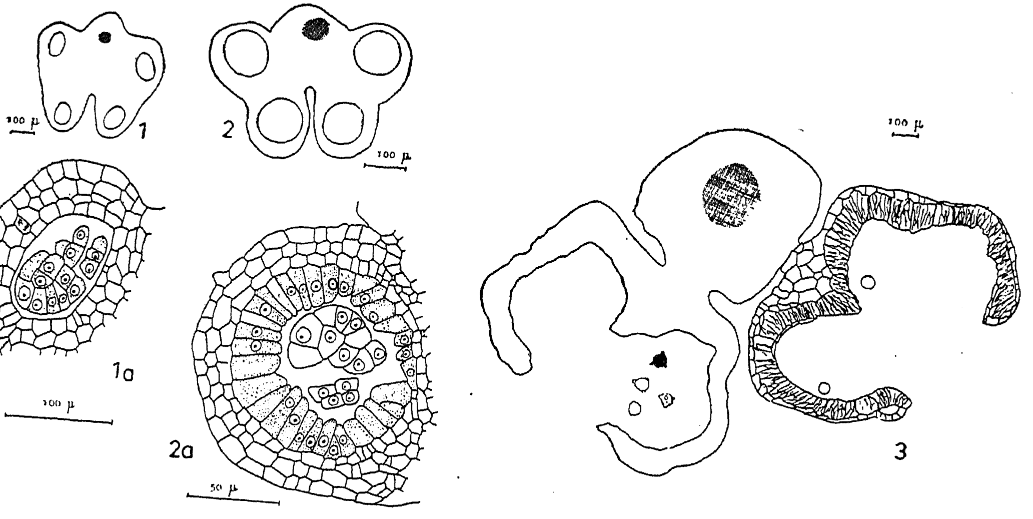
EMBRYOLOGICAL STUDIES IN THE RUBIACEAE. ORGANOGENESIS AND MICROGAMETOGENESIS IN *MORINDA CITRIFOLIA* LINN.

Morinda citrifolia Linn. is a widely growing small tree whose indeterminate inflorescence bears acropetally developing white flowers. In the peripheral rim of the floral apex, the primordia of the sepals make their appearance. The residual receptacle, which is flat apically, gives rise to the primordia of the petals. At the angles of the sinuses of the lobes of the petals, knob-like staminal primordia can be recognised. From the base of the corolla, alternating with the stamens, appear four to six elevations which grow centripetally. They meet each other and roof over the rest of the receptacle, thus constituting the ovarian chamber. The elevations continue to grow upwards, as a unit, forming the style and terminating in bifid stigma. Bicarpellary nature of the ovary becomes recognisable with the formation of the septum. A thick placental column grows into the two chambers, subsequently bearing two ovules in each chamber.

Occurrence of rod or lens-like raphides is a common phenomenon in all the floral parts.

The number of epipetalous stamens, in accordance with the other floral parts, may be four or six. Their filaments are short and anthers quadrilocular. The configuration of the anther gradually changes during development (Figs. 1, 2 and 3).

The hypodermal archesporial cells, following the pattern of development for the family[1,2],



FIGS. 1–3. *Morinda citrifolia*. Figs. 1–2. Transections of the anther (Outline diagrams). Figs. 1 *a*–2 *a*. Sectors of the anthers of Figs. 1 and 2 with details. Fig. 3. Transection of a mature anther.

differentiate into three wall layers: the outermost layer, adjacent to the shrivelling epidermis, develops fibrous thickenings and becomes the endothecium. The two cell thick middle layer and the single innermost tapetal layer enclose the sporogenous tissue (Figs. 1 *a* and 2 *a*). Endothecium persists but the middle layer gradually degenerates.

The tapetum is of a single layer of elongated cells. However, the cells, adjacent to the side of the connective, are smaller in size and not well aligned with the rest of the tapetal cells which are much elongated and the staining reaction was also slightly different (Fig. 2 *a*). This duality in the origin of the tapetal cells recalls the dual origin of the tapetum[3] and fully endorses the view that the origin of the tapetum should be studied with the help of appropriate staining supported by histo-chemical tests to understand the behaviour of these cells at successive stages of ontogeny. Following meiosis in the microspore mother cells, the tapetum exhibits a glandular or parietal type of development.

Microspore mother cells undergo successive type of meiotic division. The microspore tetrads are usually tetrahedral but decussate and isobilateral types also occur rarely.

At maturity, the male gametophytes are shed at 2-nucleate stage which is rather unusual for the family. It is also interesting to note the crystals, as mentioned in the earlier part of this paper, in the pollen grains.

The authors are thankful to Dr. V. S. Sundaralingam and the Pachaiyappa's Charities for providing all facilities.

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---

1. Davis, G. L., *Systematic Embryology of Angiosperms*, John Wiley and Sons, London, 1966.
2. Farooq, Md. and Inamuddin, M., *J. Indian bot. Soc.*, 1969, 48, 166.
3. Lakshmanan, K. K., *Phyton* (Argentina), 1963, 20, 49.

BASIDIOCARP FORMATION BY *CORIOLELLUS* SP. IN AGAR CULTURE

Formation of basidiocarp in agar culture is an uncommon phenomenon. It is recorded in some Agaricales (Agaricaceae[4,6], Clavariaceae[3,7,8] and Boletaceae[2]). Polyporales[1] produce them very rarely in agar culture. On sterilized wood pieces some polyporales are, however, known to produce basidiocarps[9]. In some species interfertility[5] has also been examined. The authors collected a species of *Coriolellus* on twigs collected from river Ganges and subsequent efforts resulted in successful culture and fructification formation by this fungus under laboratory conditions. This organism was able to grow and produce fruiting bodies on a variety of mycological media, but malt-extract medium induced best growth and size of the basidiocarp. However, the dimension of fruiting bodies was smaller in culture as compared to that produced in natural habitat. The size of pores, basidia and basidiospores were almost similar both under laboratory as well as natural conditions.

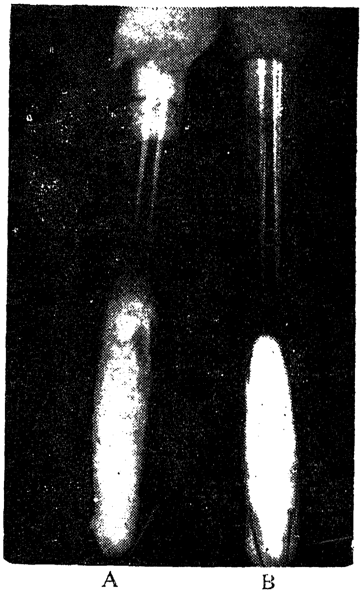

Fig. 1. A. Basidiocarp formation in malt extract agar medium. B. Without basidiocarp in Czapeck's medium.

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Bhagalpur 812 007, *February* 17, 1977.

---

1. Badcock, E. C., *Trans. Brit. Mycol. Soc.*, 1941, **25**, 200.
2. David, J. Mclaughlin, *Mycologia*, 1970, **62**, **307**.
3. — and Esther G. Mclaughlin, *Ibid.*, 1972, **64**, **599**.
4. Davis, E. E. and Jong. S. C., *Ibid.*, 1976, **68**, **211**.
5. De, A. B., *Curr. Sci.*, 1977, **46** (2), 58.
6. Goos, R. D., *Mycologia*, 1960, **52**, 661.
7. Marry Ann Dhillon and Constantine J. Alexopoulos, *Ibid.*, 1969, 61, 997.
8. Richard E. Koske and Chester E. Leathers, *Ibid.*, 1969, 61, 999.
9. Santra, S. and Nandi, B., *Curr. Sci.*, 1976, **45** (8), 308.

DIOSGENIN–DRY MATTER RELATIONSHIP IN TUBERS OF *DIOSCOREA* SPECIES

DIOSGENIN, a steroidal sapogenin, is the base chemical for the preparation of a number of pharmaceutical products. Its availability from the tubers of some of the wild species of *Dioscorea* is commercially exploited. *Dioscorea*, generally grown wild, is brought under cultivation, thanks to the research work carried out at this Institute[1]. The purchase price of wild yam tubers is fixed based on diosgenin per cent. This necessitated the estimation of a representative tuber sample for diosgenin content by the purchaser. Diosgenin estimation by chemical procedures are tedious and time consuming. An observation made earlier[2] about the relationship between the colour of fresh cut *D. deltoidea* tuber and diosgenin per cent has helped in rapid screening of tuber samples. It was not possible to arrive at such relationship in *D. floribunda*, because the freshly cut tuber colour is mainly yellow. Correlations, if any, between the diosgenin and other easily estimable constituent can also help in the satisfactory assessment of tuber samples. With this idea in view, correlations[3] were worked out between diosgenin and dry matter and the results are reported. Diosgenin was estimated according to the procedure described earlier[4] and dry matter by standard AOAC[5] method.

The data used were gathered during the analysis of *D. floribunda* tuber samples for diosgenin from various treatments and replications of the field experiments conducted during the year 1972 to 1976. The data on the screening of clones received for introduction at this centre from various regions of India were used in the case of *D. deltoidea*.

Table I gives the data of diosgenin dry matter correlation coefficients. In one year old tubers of *D. floribunda*, the relationship was significant but with low prediction index. Attempts to improve correlation coefficients by taking log dry matter, in fact, decreased the correlation. Even when the data were split into different diosgenin classes the correlation coefficients did not show any improvement. However, the correlation coefficients improved substantially for two year old tubers, showing that in one year old tubers the relationship does not attain a state of equilibrium and is perhaps in a state of flux. The improved relationship in a two year old tuber is quite interesting from the point of view of predicting the diosgenin content. The tuberisation rate is very slow in *D. deltoidea* and the samples collected may be of more than two year old. The existence of relationship shows that the two year period may be quite valid even for *D. deltoidea*.

The relationship between diosgenin and dry matter in both species provides an easy, inexpensive method for predicting the diosgenin content, to a reasonable extent (Table I).

TABLE I

Correlation coefficients between diosgenin and dry matter

Category of sample	Correlation of diosgenin (on fresh weight basis) with	Number of observations	Correlation	Regression equation for diosgenin	S.E. ($\hat{Y}$ estimated)
D. floribunda (one year old)	dry matter	200	0·4101**	0·4620+0·2257 dry matter	0·729
D. floribunda (one year old)	log dry matter	200	0·3013**	1·3937+0·0769 log dry matter	0·763
D. floribunda (Two year old)	dry matter	60	0·6562**	−0·16157+0·03455 dry matter	0·187
D. deltoidea (age not known)	dry matter	174	0·5805**	2·2913+0·06974 dry matter	0·274

** Significant at 1% level.

We are thankful to Dr. T. R. Subramanian, Senior Soil Scientist and Shri P. R. Ramachander, Statistician, for their suggestions and help in the statistical analysis.

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---

1. *Status Report, Dioscorea Improvement Project* (Published by Indian Institute of Horticultural Research, Bangalore, March 1975.

2. Bammi, R. K., Selvaraj, Y., Sobti, S. N., Randhawa, G. S. Subhas Chander, M. and Rama Rao, V., *Indian J. Hort.*, 1972, **29**, 313.
3. Snedecor, G. W. and Cochran, W. G., *Statistical Methods*, Oxford and IBH Publishing Co., New Delhi, 1967, p. 172.
4. Selvaraj, Y., *Indian J. Hort.*, 1971, 2[illegible], 135.
5. A.O.A.C., *Official Methods of Analysis*, Association of Official Agricultural Chemists, Washington D.C., 1968.

PHYTOTOXIC METABOLITE FROM *ALTERNARIA MACROSPORA*

THE leaf spot disease of cotton (*Gossypium hirsutum*) caused by *Alternaria macrospora* is a widespread and destructive disease that has affected many areas in Southern India[1]. Alternariosis is characterized by irregular necrotic lesions on leaves. The syndrome can be reproduced by artificial inoculation and also by the culture filtrate on leaves. Phytotoxic metabolites produced by this organism *in vitro* from 7–10 day old cultures grown on Richards' medium when tested using the method of Ueno *et al.*[2] (1975) showed toxicity to susceptible cotton leaves (MCU 5). The brown, necrotic lesions appeared after 16 h of treatment enlarging within 24 h resulting in veinal necrosis. Affected cell walls of the subsidiary cells turned brown. The rapidity of appearance of the phytotoxic symptoms was greater when the organism was grown in potato sucrose broth for 15 days than in Richards' medium. Culture filtrates were extracted with ether and further processed into acid, neutral and alkaline fractions as suggested by Kohmoto *et al.*[3] (1976). Each fraction was partially purified by preparative TLC using benzene : acetone (2 : 1) as the solvent system. As many as 15 zones were partitioned under UV and tested for phytotoxicity. Between the zones, there was an UV absorbing one present in all the three fractions which was found to be most toxic. This partially purified substance has an R_f value of 0·74 to 0·76. The silica gel containing the sample was then scraped off the glass plate and placed in a small circle on the upper side of the fresh detached leaf (MCU 5) on moist filter paper in a Petri dish. The silica gel on the leaf was wetted with distilled water and incubated in a moist chamber at 27° C. The leaf was examined for the induction of veinal necrosis after 16–24 hours (Ueno *et al.*[2], 1975). Droplets of water with plain silica gel served as the control. Also, leaves of *Cyamopsis tetragonaloba* and *Phaseolus vulgaris* produced the same syndrome. This fraction gave positive reaction (characteristic blue colour) with Folin's reagent when followed by exposure to ammonia vapour, suggesting that this might be a phenolic acid. Further studies on the chemical nature as well as its action on the permeability of epidermal cells are underway.

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University Botany Lab., Madras-5,
April 4, 1977.

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K. S. BHAMA.

1. Srinivasan, K. V. and Kannan, A., *Curr. Sci.*, 1974, 43, 489.
2. Ueno, T., Hayashi, Y., Nishimura, T., Fukami, H., Nishimura, S., Kohmoto, K. and Sekiguchi, S., *Phytopathology*, 1975, 65, 82.
3. Kohmoto, K., Khan, I. D., Renbutsu, Y. Taniguchi, T. and Nishimura, S., *Physiol. Plant. Pathol.*, 1976, 8, 141.

AWARD OF RESEARCH DEGREES

Tamil Nadu Agricultural University, Coimbatore, has awarded the Ph.D. degree to Messrs. S. Subrahmanyan, M. Nagarajan, T. S. Manickam, B. Sethupathi Ramalingam, K. M. Ramanathan.

Utkal University, Bhubaneswar, has awarded the Ph.D. degree in Chemistry to Shri Panchanan Patra.

Karnatak University, Dharwar, has awarded the Ph.D. degree in Geology to Shri K. T. Damodaran.

Karnatak University, Dharwar, has awarded the Ph.D. degree in Mathematics to Shri Rajasekharan Mohansingh.

Kakatiya University, Warangal, has awarded the Ph.D. Degree in Chemical Engineering to Shri T. Sripathi.

University of Cochin, has awarded the Ph.D degree in Science to Smt. B. Santhakumari.

REVIEWS AND NOTICES OF BOOKS

[illegible]tegral Geometry and Geometrical Probability. [illegible]

[illegible] M[illegible] K[illegible]

[illegible]

As Mark K[illegible] [illegible] geometry." [illegible]

[illegible] Addison-Wesley [illegible] Professors [illegible] K[illegible] [illegible]

J. N. K[illegible]

Stochastic Processes. [illegible] K. M. [illegible] McGraw-Hill Publishing Co. Ltd., New Delhi [illegible] Pp. [illegible] Price Rs. 24.00.

[illegible] Preface that "The emphasis [illegible] to present a [illegible] model building [illegible] attempt has been made to keep the [illegible] mathematical techniques to the minimum". [illegible] point of view and nobody can take [illegible] However, this does not justify [illegible] has the right to expect that in [illegible] consideration of a situation, the mathematical difficulties [illegible] should be pointed out even [illegible] are suggested.

[illegible] from the table of contents, one gets the impression that the book covers a variety of topics of interest in applications, and this is by [illegible] borne out of the text material. The [illegible] criticism is that the book is carelessly written and this is all the more surprising [illegible] the claim made that the book largely [illegible] out of a series of lecture courses. The authors do not seem to be able to decide whether a probability is positive or non-negative; on p. 8 one reads: "a function P(·) which assigns to every Borel set E... a non-negative real number denoted by P(E) according to the following axioms: 1. P(E) > 0 for every event E." This ambivalence persists right through. On the same page it is asserted that every probability function has a density. On p. 10, X^{-1} is called the inverse function of X while the inverse map may not exist. Again on p. [illegible] one is told that the "probabilisable sets" on the line are "nothing but intervals" and the next sentence reads: "It can be shown that there always exists a smallest family of probabilisable sets on the real line which form a Borel field." It is unfortunate that such contradictory statements which cannot but confuse a beginner occur in the first chapter. A howler of the first magnitude occurs on p. [illegible] where the singular component in the Lebesgue decomposition of a non-decreasing function is defined as "a continuous function which is constant almost everywhere". There are numerous other instances of slipshod statements. One cannot object if in a particular mathematical situation, the authors content themselves with a general statement that mathematical difficulties can be overcome, but when they specifically suggest remedies, these have to be given precisely. In spite of these adverse comments, the book could be useful if read with proper care.

The printing of the book was done by a press which apparently does not have a trained proof-reader. Almost every page contains a printing mistake; to be sure, often these are errors in punctuation which, however, can be very mystifying at times. The reviewer noticed at least one instance where whole lines of the text are omitted. The printers do not seem to be aware that mathematical printing has its own aesthetics; they could have learned something about this if only they had cared to look at McGraw-Hill Company's own mathematical publication.

K. BALAGANGADHARAN.

Reaction Mechanisms in Organic Chemistry. By S M. Mukherji and S. P. Singh. (Macmillan Company of India Limited, New Delhi 110 002), 1976. pp. 388 + vi. Price Rs. 24.00.

The authors have attempted to write a concise introducion to reaction mechanisms, meant primarily for post-graduate students of the Indian Universities. The authors have succeeded in producing a book, impressive in get-up, moderate in price and generally free from trivial errors.

In the fourteen chapters, the authors cover many of the subjects ordinarily expected to be dealt with in such a book. One might question the inclusion or omission of certain topics here and there, but a certain amount of subjectivity is unavoidable. The first chapter covers "the chemical bond" and later chapters go on to describe the stock-in-trade of reaction mechanisms. The last two chapters are on organic photochemistry and pericyclic reactions.

The industry and effort the authors have bestowed on interpreting difficult concepts, to make them more intelligible, is evident throughout the book. One is however disappointed to find that the authors do not give references. Also one looks in vain for the flavour and freshnes of the original. In some chapters one comes across material hopelessly out of date as to be misleading. Consider a few examples:

P. 28; "Sydnones are stable aromatic compounds". In fact by most criteria they are not considered aromatic.[1]

p. 28: "The aromatic inorganic compounds having no carbon at all......" Again these compounds are not deemed aromatic[2].

Pp. 216–218: "Ring-chain tautomerism"—A large part of the material discussed here has subsequently been shown to be incorrect[3/4].

Also normal phthaloyl chloride does not exist in equilibrium with pseudo phthaloyl chloride.

P. 309: Triphenylmethyl radical does not gi hexaphenylethane as reported here. Later findin show that the reaction gives different products[5].

There are also a few errors of fact and interpretati Following are examples:

P. 1: "Lewis structures......were later modifie by Couper", G. N. Lewis (1875–1946)[7], A. S Couper, (1831–1892)[6].

P. 30: "Cyclo octatetraene however can escap as it does, its antiaromaticity by abandoning its plan geometry".

Factors nothing to do with aromaticity or an aromaticity may be responsible for its nonplanari cf. cyclo-octene, cyclo-octadiene, and cyclo-octatri are non-planar[8].

There are some examples of incorrect or inad quate expression or plain Indianism.

P. 31: "Conjugated hydrocarbons may be......" "Conjugated *aromatic* hydrocarbons......".

P. 29: "One of the spectacular examples of Hucke rule is the discovery......"

Ideas are jumbled.

P. 29: 4*q* annulenes are not "neighbours" of 4 +2 annulenes. These deficiencies (which can b corrected in a subsequent edition) should not blin us to the fact that authors have written a reasona good book which can be useful to M.Sc. studen

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1. Garret, P. J., *Aromaticity*, McGraw-Hill Bo Company, U.K. Ltd., 1971, p. 135.
2. Allcock, H. R., *Phosphorus-Nitrogen Co pounds*, Academic Press, N.Y., 1972, Chapte I and III.
3. Larson, H. O. and Sung, G. S. K., *Austral. J Chem.*, 1962, **15**, 261.
4. Wiberg, K. B. and Holmquist, H. W., *J. O Chem.*, 1959, **24**, 578.
5. Staab, H. A., Brettstneider, H. and Brunner, H. *Chem. Ber.*, 1970, **103**, 1101.
6. Gillispie, C. C., *Dictionary of Scientific Bio graphy*, Charles Scribners Sons, N.Y., 197 3, 448.
7. —, *Ibid.*, 1973, 8, 289.
8. Lippincott, E. R., Lord, R. C. and McDonal R. S., *J. Am. Chem. Soc.*, 1951, 73, 3370.

M. V. I

Annual Review of Genetics, Vol. 10. Edited b Herschel L. Roman. (Annual Reviews, In Palo Alto, California), Pp. 465. Price: USA $ 17.0 Elsewhere $ 17.50.

Volume 10 of this series lives up to the usual st dards of the *Annual Review*, and the reviews clea show the insight and a willingness of the authors be informative and provocative.

There are altogether fifteen essays in Vol. ranging from plasmids, insertion elements of bact to developmental and biochemical genetics of Dro phila and human genetics.

A. M. Chakrabarty describes the nature and properties of plasmids in *Pseudomonas*. This includes the study of the degradative plasmids which are a new type, helping in the survival of the organism in soil by their nutritional versatility, *i.e.*, capacity to eat up hydrocarbons of oil and resistance to mercury salts. This review is very rewarding in view of the rapid emergence of drug-resistant strains of *Pseudomonas* which is a major clinical problem and is important in the utilization of hydrocarbons.

MacIntyre and O'Brien have compiled very useful data on how biochemical loci are detected and mapped in Drosophila, which is fast becoming a popular organism for the investigations in molecular genetics.

Weatherall and Clegg have reviewed the current state of knowledge about the molecular genetics of human hemoglobin. This gives a clear insight about the knowledge on the normal genetic control of hemoglobin production at the molecular level and projects questions which are unanswered, such as, control of erythroid differentiation and molecular basis for β-thalassemias.

Transposition elements, popularly known as "jumping genes" have recently assumed great importance in genetics. A. I. Bukhari deals extensively with one of these transposition elements, *viz.*, bacteriophage, Mu. More details of such elements are available in a recent monograph, "DNA Insertion Elements, Plasmids and Episomes" published by Cold Spring Harbor Laboratory, New York.

Other topics covered in the Reviews include "Genetic Control of Chromosome Pairing in Wheat" by E. R. Sears, "The Genetic Control of Meiosis" by Baker *et al.*, "Bacterial Mutator Genes and Control of Spontaneous Mutation" by DeFries *et al.*, "The Theoretical Population Genetics of Variable Selection and Migration" by J. Felsenstein, "Genetics of Transplantation" by F. H. Bach and "Genetics of the Bacterial Phosphoenolpyruvate: Glycose Phosphotransferase System" by C. Cordaro.

M. S. SHAILA.
T. RAMAKRISHNAN.

Irrigation and Drainage. By R. K. Sivanappan and K. R. Karai Gowder. Popular Book Depot, 14–B, Sterling Road, Nungambakam, Madras 600034, 1977, Pp. 308, Price Rs. 20–00.

Even though the book is written primarily for undergraduate students in Agricultural Engineering, Soil Physicists and Irrigation agronomists will find this book to be an excellent source for complete information on irrigation and drainage. This book is a welcome edition to the subject of irrigation and drainage. The book is well organized and illustrated.

This new book has the following chapters:

(1) Introduction, (2) Sources of irrigation water, (3) Wells and well sinking, (4) Water requirement of crops, (5) Conveyance and distribution of water, (6) Land levelling and grading, (7) Measurement of water, (8) Soil-water-plant relationship, (9) Scheduling irrigation, (10) Surface and subsurface irrigation. (11) Sprinkler irrigation, (12) Irrigation of important crops, (13) Agricultural drainage and reclamation, (14) Design, construction and maintenance of drainage, and (15) Water lifting devices for irrigation and drainage.

The different sections of the book that deal with actual case studies and with practices are of unique value. All the various kinds of efficiencies associated with irrigation are listed and defined. Procedures for estimating hydraulic conductivity, infiltration rate, etc., are presented including plans for much of the equipments used.

The book contains 25 tables and more than 100 figures. Much of the data presented in the figures should be of particular value to the teacher. There are 111 impressive literature citations. However, the majority of references are in the pre–1970 with approximately less than 5% of the references in the 1970–75 era even though the book is published in 1977. In 36 citations, year of publication has not been given and is really a glaring mistake. In some cases, references have not been given in chronological order, *e.g.*, Pages 134, 229 and 253.

The authors cover a wide range of subjects and possibly because of this, certain subjects are treated in a very superficial manner. The information such as net area irrigated, area irrigated source-wise, etc., given in tables are old and should have been 1975 later data. The chapter on soil-water-plant relationship is incomplete. Soil-water has been discussed in brief but not on water-plant relationship. Pressure plate apparatus comes handy in determining 1/3 and 15 bars moisture but has not been included. Though the title of the book is 'Irrigation and Drainagee, the drainage part has not received the same attention of the authors as that of the irrigation part. The book is not free from spelling mistakes.

Though the price is reasonable, the authors should have used better quality paper for printing and the book should have been well bound. The present publication has filled the lacuna to a great extent and the publication serves as a useful text for the undergraduate students in Agricultural Engineering and Agriculture.

T. SATYANARAYANA.

6485/77. Published by S. R. S. Sastry, Manager, Current Science Association, Bangalore-560006 and Printed at The Bangalore Press, Bangalore-560018.

A CRYSTALLOGRAPHIC STUDY OF SEMICONDUCTOR-METAL TRANSITIONS IN PURE AND V-DOPED Ti_2O_3

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AND
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ABSTRACT

Variations of lattice dimensions, Ti–Ti distances and other structural parameters of Ti_2O_3 with temperature and with V-doping are reported. The results are consistent with the band crossing mechanism of the semiconductor-metal transition.

Ti_2O_3 undergoes a semiconductor-metal transition around 400° K without any accompanying change in crystal symmetry[1,2]. The transition is, however, accompanied by a change in the c/a ratio. The transition is considered to be due to the crossing of a_1 and e_π bands with increase in temperature[3]. All the recent studies on the Ti_2O_3 transition are in conformity with this mechanism. According to this mechanism, the Ti-Ti distance along the three-fold axis should increase with temperature, while the opposite would be true for the Ti-Ti distance in the basal plane. Doping of Ti_2O_3 by V_2O_3 progressively reduces the magnitude of the conductivity jump, until at 10% V_2O_3, the material is metallic[4]; the lattice parameters of 10% V_2O_3-doped sample are reported to be close to those of the high temperature metallic phase of Ti_2O_3[5,6]. We have been carrying out detailed crystallographic studies of pure Ti_2O_3 and also of Ti_2O_3 doped with V_2O_3 for the past some time. We have just noticed a publication of Rice and Robinson[7], on the temperature-dependent structural changes in pure Ti_2O_3. The results of these workers are entirely in agreement with our results[8] in that both the studies show the expected variations in Ti–Ti distances and other structural parameters with temperature. In this communication, we report the results of our detailed crystallographic studies on the V_2O_3-doped Ti_2O_3 system. We consider these studies to be important in understanding the mechanism of the semiconductor-metal transition in Ti_2O_3 and the mode of action of the V^{3+} impurity.

TABLE I

Lattice parameters and structural data of V_2O_3-doped Ti_2O_3

	Ti_2O_3	0·5% V_2O_3	2·0% V_2O_3	4% V_2O_3	10% V_2O_3	H.T. Ti_2O_3 (580° K)
a Å	5·431 (1)	5·431 (1)	5·439 (1)	5·451 (4)	5·473 (1)	5·478 (2)
α deg	56·71 (1)	56·627 (1)	56·37 (1)	56·07 (2)	55·57 (1)	55·84 (1)
a_{hex} Å	5·159 (2)	5·155 (2)	5·138 (1)	5·125 (3)	5·102 (1)	5·129 (2)
c_{hex} Å	13·625 (2)	13·629 (2)	13·677 (3)	13·735 (3)	13·840 (3)	13·823 (2)
c/a	2·641	2·644	2·662	2·680	2·713	2·695
Bond Length Å						
M_1–M_2	2·582 (1)	2·584 (0)	2·602 (0)	2·628 (0)	2·658 (0)	2·657 (0)
M_1–M_3	2·994 (0)	2·989 (0)	2·982 (0)	2·976 (0)	2·968 (0)	2·982 (0)
M_1–O_1	2·067 (1)	2·061 (2)	2·067 (1)	2·070 (2)	2·072 (1)	2·079 (1)
M_1–O_5	2·027 (0)	2·027 (1)	2·019 (0)	2·017 (1)	2·012 (1)	2·019 (1)
O_1–O_2	2·796 (1)	2·787 (2)	2·782 (1)	2·769 (2)	2·753 (1)	2·769 (1)
O_4–O_5	3·073 (1)	3·074 (1)	3·060 (1)	3·056 (1)	3·048 (1)	3·062 (1)
O_1–O_4	2·792 (1)	2·787 (2)	2·794 (1)	2·801 (2)	2·805 (1)	2·811 (2)
O_1–O_1	2·882 (2)	2·882 (3)	2·884 (2)	2·894 (3)	2·899 (2)	2·904 (2)

Single crystals of Ti_2O_3 and V_2O_3-doped Ti_2O_3 were mounted on the goniometer head of an Enrauf-Nonius CAD-4 diffractometer. A high temperature attachment built locally was used for the study of Ti_2O_3[9]. Lorentz polarisation and absorption corrections were applied and the structures refined by least-square treatment.

Variations of the lattice parameters of Ti_2O_3 with temperature and also with the incorporation of V_2O_3 are shown in Fig. 1. We see from the figure that the lattice parameter variations in the two cases are not exactly identical. Thus, the unit cell volume of Ti_2O_3 increases with tempera-

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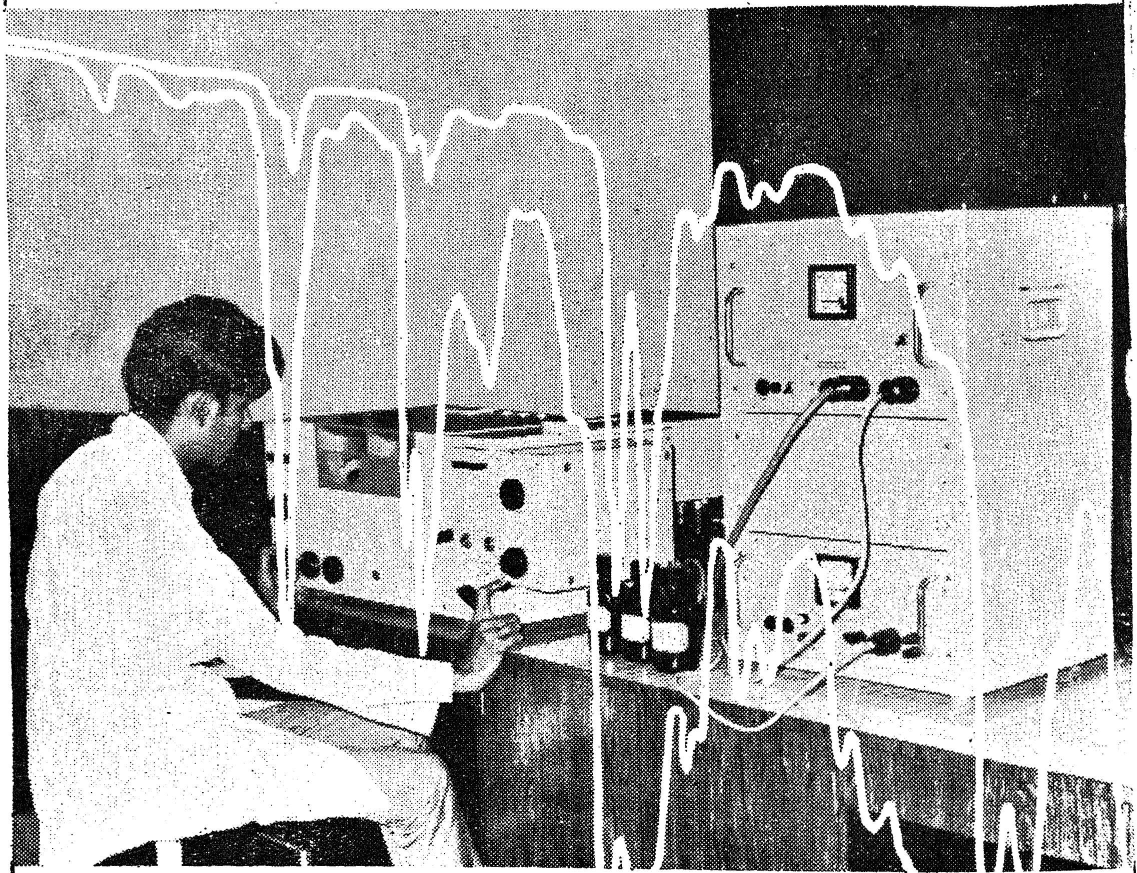
solvents for spectroscopy



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tion during the early part of the night is of Range type while the Frequency spread is generally a post-midnight phenomenon.

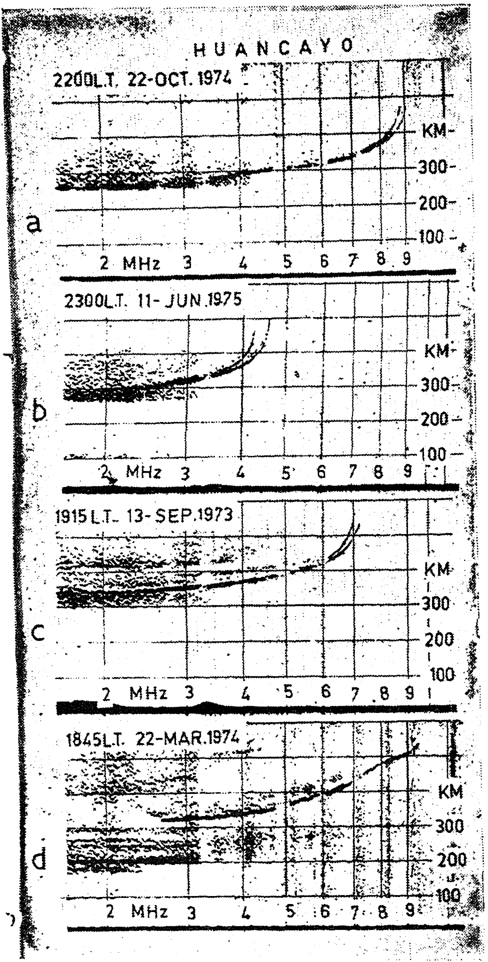

FIG. 1. A few ionograms at Huancayo showing Range-spread F simultaneously with clear h'–f trace.

RESULTS

In Fig. 1 are shown a few sample ionograms of Huancayo with Range-spread F configuration. The ionogram in Fig. 1 (*a*) is the generally common type of Range-spread in which the critical frequencies are very clear and the diffuse echoes are seen only at the lower frequency end of the ionogram. The normal h'–f trace cannot clearly be seen in these conditions within the spread F echoes. The virtual heights of the normal trace and the spread F echoes cannot be differentiated in most of these cases. In Fig. 1 (*b*) is shown the ionogram at 2300 LT on 11 June 1975. Here too the critical frequencies are very definitely identified. The spread F echoes have a clear minimum virtual height which is more or less constant with frequency. Within the frequency range 2–3 MHz the minimum height of the spread is lower than the virtual height of the normal echoes; however, it is difficult to be sure if the height of the spread F echoes is less than the base of the F layer. In Fig. 1 (*c*) is reproduced the ionogram at 1915 LT on 13 September 1973 where the normal F region trace can be seen below 2 MHz even within the spread F configuration. The spread F is seen from heights significantly below the base of the F layer. A more remarkable ionogram is shown in Fig. 1 (*d*) at 1845 LT on 22 March 1974. In this case the spread F echoes are seen even at the height of about 180 km whereas the minimum virtual height of the F layer is more than 300 km. The trace of the ionogram being fairly constant with frequency, the base of the F layer would not be much different from h'F. This record clearly demonstrates that the Range-spread F starts at a height significantly lower than the base of the F layer sometimes by more than 100 km.

Further close examination of the spread F configuration suggests a number of regions of constant height from where the scattering occurs. It is suggested that these spread F layers below F layer are due to large plasma density gradients present between the E and F regions during night-time hours.

ACKNOWLEDGEMENT

Thanks are due to U.S. Academy of Sciences for the Arthur L Day Grant No. 30 for an intensive study of equatorial spread F. Thanks are also due to Dr. Alan H. Shapley for his cooperation in this investigation. The research program at PRL is supported by the Department of Space, Government of India.

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1. Chandra, H. and Rastogi, R. G., *Ann. de Geophys.*, 1972, 28, 37.
2. Clemsha, B. R. and Wright, R. W. H., "A survey of equatorial spread F," In *Spread F and Its Effects Upon Radio Wave Propagation*, ed. by P. Newman, W. and J. Mackay and Co., Ltd., London, 1966, p. 3.
3. Cohen, R. and Bowles, K. L., *J. Geophys. Res.*, 1961, 66, 1081.
4. Rastogi, R. G., *Proc. Ind. Acad. Sci.*, 1977, 85 A (4), 230.

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TONSILS AND OTHER LEUCOCYTOPOIETIC CENTRES IN THE INDIAN WATER-SKIPPING FROG *RANA CYANOPHLYCTIS* (BOULENGER)

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ABSTRACT

Frogs are very prone to infections of bacteria and the protozoa as they imbibe water through their skin or breathe air into their lungs. They are also liable to attacks by similar microbes in the soil during hibernation. Therefore their bodies are equipped with special leucocytopoietic centres to fight such infections. During active periods these centres produce leucocytes, at other times they are reduced to mere adipose tissues.

INTRODUCTION

AMPHIBIAN tonsils were reported by Kingsbury[5], Braunmuhl[1], and Knower[4] and quite a few lymphoid and leucocytopoietic centres were described, some of which were associated with blood vessels. This author[6] (1975) described tonsils and a number of leucocytopoietic centres in the Indian bullfrog *Rana tigrina* (Daud). Since then the same author has been working on other Indian anura. This paper is aimed to describe the tonsils and leucocytopoietic centres in another Indian species of frog.

MATERIAL AND METHOD

Freshly narcotized and formalin preserved frogs of this rather less-known and small-sized species were dissected (throughout the year) taking into account both active and hibernation periods. Gum was injected[4] into the lymphatic system through the dorsal cutaneous lymph sinus of frogs narcotized with 3 ml of 1% urethane solution. The injection increased the colloidal osmotic pressure in the tissue spaces; this prevented the back flow of the fluid into the venules and hence led to the swelling of lymph sinuses. This technique helped in observing the lymph sinuses and studying the lymphoid centres. The suspected lymphoid centres were exposed and their relations with the blood vessels traced out. Grafts from the suspected centres were macerated on the microslides and examined for their histological nature and contents. Leishman's stain was used to identify the leucocytes.

The lymphoid centres appeared as distinct irregular but well organized pale yellow or pinkish masses in the preserved as well as fresh frogs (collected from February to September).

OBSERVATIONS

1. *Buccal or preorbital tonsils.*—These organs were exposed by cutting away the roof of the mouth cavity, where they appeared as two symmetrical, pinkish, oval and compact structures in front of the inbulgings of the eye balls and behind the vomerine teeth. It may be recalled that *Rana tigrina* and pharyngeal tonsils placed just beneath the inbulgings of the eye balls. Because of their forward positions in *Rana cyanophlyctis,* they have been designated as 'buccal tonsils' or preorbital tonsils'.

2. *Thymus.*—This is a lymphoid organ. A pair of these was observed behind the tympanic membranes and under the depressor mandibulae muscles.

3. *Superior and inferior pectoral leucocytopoietic centres.*—These paired and bilaterally symmetrical centres were identified by their yellowish tinge in the vicinity of the pectoral girdle lying superficially between the skin and the muscles. The superior pectoral centre was placed in front of the clavicle and was exposed by lifting the pectoral and coracoidal muscles overlying it. The centre was closely associated with the faciomandibular vein where it split up into facial and mandibular veins. The entire centre was bathed by the large pectoral lymph sinus. The inferior paired centres were exposed by cutting away the skin from the chest and lifting the pectoral muscles to bring in view the coracobrachial muscles. The roughly triangular inferior centre, like the superior centre, was inter-spread at the junction of two veins, brachial vein from the arm and the musculocutaneous from the abdominal muscles and the skin. Contrary to the situation in *Rana tigrina,* these centres were not extended into the arms, but likewise they were bathed by the large pectoral lymph sinus.

4. *Pericardial or median pectoral centre.*—This occupied the same position as in *Rana tigrina,* lying in front of the heart and behind the hyaline omosternum of the pectoral arch. However, this centre was proportionately bigger than the same in *Rana tigrina* and was bilobed having orange-yellow colour and somewhat lobular in nature.

5. *Internal mandibular centre.*—This paired centre was approached by first cutting away the skin of the throat thereby exposing the large mylohyoid muscle below the floor of the mouth cavity, and by further pulling out the same muscle,

Each mandibular centre appeared as yellowish, broad with somewhat pleated inner border parallel to the mandibular ramus. It surrounded the internal mandibular vein all the way up to the tip of the snout. It was bathed by the sub-maxillary lymph sinus.

6. *The sublingual centres.*—These were exposed along with the internal mandibular centres as two closely set ovoid and compact bodies of yellowish colour (somewhat diffused in *Rana tigrina*).

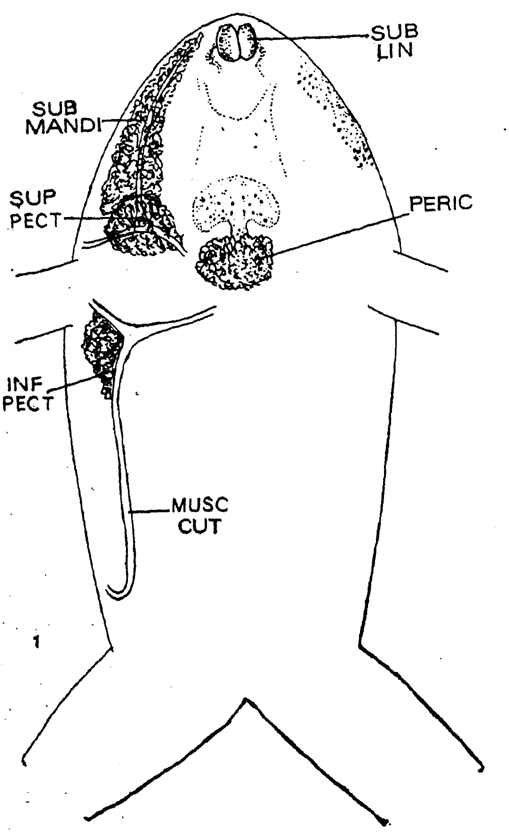

Fig. 1. Diagram of the leucocytopoietic centres of *Rana cyanophlyctis* (ventral), × 3. (SUB LIN, sublingual centre ; SUB MANDI, submandibular centre ; SUP PECT, superior pectoral centre ; INF PECT, inferior pectoral centre ; PERIC, pericardial centre ; MUSC CUT, musculocutaneous vein.)

Discussion

Presence of lymphoidal masses in the body of anura was known[7] long before this author[6] described them in the Indian bullfrog *Rana tigrina*. Most of the lymphoid centres were found in association with larger veins or between the skin and the muscles, some were actually surrounded by lymph sinuses. It was confirmed[1] that during spring, these lymphoid masses produced both lymphocytes and phagocytes, but appear to store fat at other seasons. From the fact that lymphocytes are accumulated in the lymphoid centres during the active periods of the lives of the frogs, some workers[5,9] named at least one pair of lymphoid organs as 'tonsils'. The present author found tonsils earlier in *Rana tigrina* and now in *Rana cyanophlyctis*, but in different locations as referred to earlier in the text. Histological preparations of the tonsils and other leucocytopoietic centres revealed that during active periods of *Rana cyanophlyctis*, (*i.e.*, March to September) these centres are leucocytopoietic and crowded with leucocytes, the adipose cells are scanty.

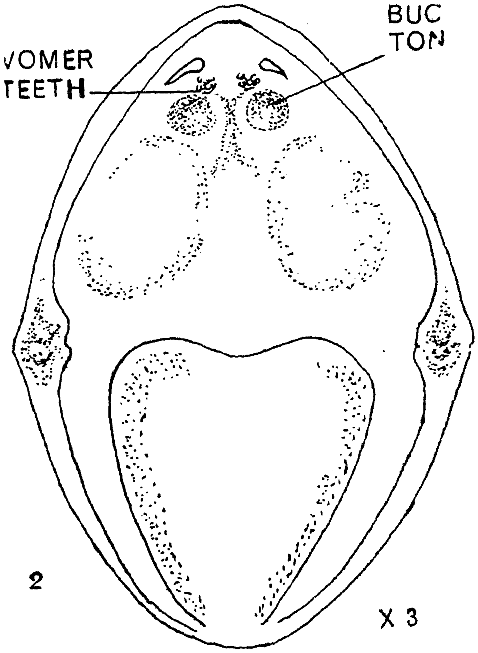

Fig. 2. Diagram of the mouth cavity of *Ra* *cyanophlyctis* showing the location of the buc tonsils, × 3. (BUC TON, buccal tonsil ; VOMI TEETH, vomerine teeth.)

From middle October to following February, t adipose cells take over the leucocytes whi become far less in number than the adipose ce (Plates I and II). Thus their leucocytopoi activity coincided with the active period of life of the frog.

Since most of the centres described are associa with veins, it is quite logical that the leucocy produced at these centres must be passing on i the veins and also into the surrounding lyn sinuses where they are useful in fighting the microbial infection entering through the skin.

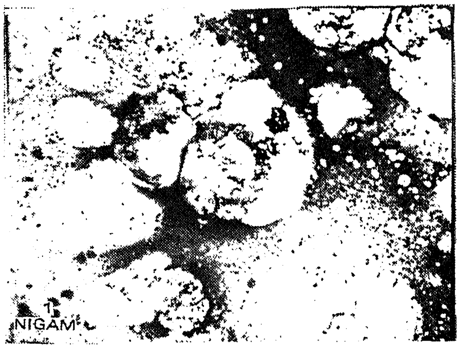

PLATE I. Photomicrograph of the leucocytopoietic tissue of the hibernation period, × 300.

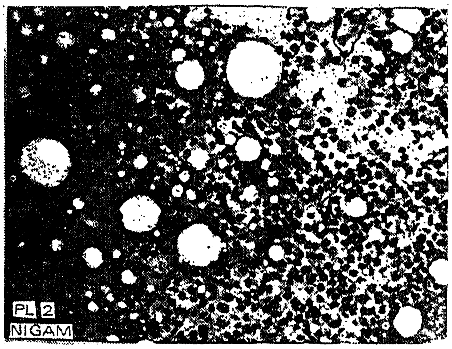

PLATE II. Photomicrograph of the leucocytopoietic tissue of the posthibernation period, × 300.

Absence of the leucocytopoietic centres in the pelvic region of *Rana cyanophlyctis*, contrary to *Rana tigrina*, may be accounted for, by the small size and slender body of *Rana cyanophlyctis*, which is roughly 1/3rd the dimensions of the bullfrog *Rana tigrina*. It is, therefore, concluded that the leucocytopoietic centres of *Rana cyanophlyctis* are also defence stations from which hosts of phagocytic cells are mobilized for attack against bacterial and protozoan invaders with the food and air reaching the mouth and lungs and also those coming with water diffusing through the skin.

1. Braunmuhl, A., von., *Zeitsch. Mikr. Anat. Forsch.*, 1926, 4, 635.
2. Bhaduri, J. L., Biswas, B. and Das, S. K., *Jour. As. Soc. Bengal*, 1933.
3. Kenyes, G., "Thymus," *Ann. Roy. Coll., Surg. England*, 1953, 12, 88.
4. Knower, H. McE., *A Resurvey of the Development of Lymphatics and Associated Blood Vessels in Anuran Amphibian Method of Injection*, Wistar. Inst. Philadel., 1939, p. 125.
5. Kingsbury, B. F., "Amphibian Tonsils," *Anat. Anz.*, 1912, 42, 593.
6. Nigam, H. C., *Curr. Sci.*, 1976, 45, 509.
   —, *Ibid.*, 1975, 44, 810.
   —, *British Journal of Herpetology* (Accepted July 1976).
   —, *Zoology of Chordates*, S. Nagin and Co., Jullundur, India, 1977.
7. Noble, G. K., *The Biology of Amphibia*, Dover Publ., 1954.
8. Moore, J. A., *Physiology of the Amphibia*, Acad. Press, N.Y., 1964.
9. Myers, M. A., *Jour. Morphol. Physiol.*, 1928, 45, 399.
10. Weichert, C. K., *Anatomy of Chordates*, McGraw-Hill Co., 1958, p. 233.

ON THE UPTAKE OF COPPER(II) BY *MERETRIX CASTA* (CHEMINITZ), AN INDICATOR SPECIES OF METAL POLLUTION

P. N. KRISHNAN NAMBISAN, P. T. LAKSHMANAN AND K. Y. MOHAMMED SALIH
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ABSTRACT

The bivalve, *Meretrix casta* (Cheminitz), when maintained in sea water containing various concentrations of copper, was found to take up large amounts of the metal; a linear relation between rate of uptake and copper (II) concentration was observed. Gill tissues form the major site of accumulation. Mortality was comparatively low, even in 10 ppm solutions.

INTRODUCTION

IN recent years, many papers on the toxicity of heavy metals on marine and estuarine organisms have been published[1-10]. It is generally observed that the metals at lethal and sub-lethal levels are accumulated to very high levels, often depending on the animals and environmental parameters[2,5,6,9,10]. However, the mechanism of such accumulation is not well understood.

Molluscs form a particularly important group of animals noted for their ability to take up large amounts of metal ions from solutions. The trace metal accumulation by estuarine molluscs has been studied by various workers[11-15]. Bivalves have also been considered as possible indicators of marine pollution by non-radioactive trace metals[9,12,14].

The enrichment of trace metals within certain animals and their transfer through food chain to higher animals constitute a danger to man. High amounts of zinc, cadmium and copper would cause symptoms of nausea and vomiting; the toxic effects of mercury are well known[15].

The present work is on the toxicity and uptake of copper(II) on the back-water clam, *Meretrix casta,* which is abundantly found in the Cochin estuary. Estuary is an important part of marine environment and owing to increased industrial activities and failure to take sufficient safety measures, it is becoming polluted by various chemicals. This study was undertaken to know the toxic effect of copper salt on the animal and to find out whether it could be used for monitoring the heavy metal pollution. The uptake of Cu by the clam, *Meretrix casta,* exposed to different concentrations of Cu was investigated.

MATERIALS AND METHODS

The clams were collected from a place about two kilometers south-east of the Cochin bar-mouth. Animals of length 30·0 ± 0·5 mm were taken. They were acclimatised to the laboratory conditions by maintaining in millipore filtered sea water in large polythene vessels; the salinity was *ca.* 10‰ which corresponds to the natural habitat salinity of the experimental clams. The water was aerated daily. Two series of tests were conducted.

In case 1, thirty animals each were kept in five litres of sea water containing 0·5, 1·0, 5·0 and 10·0 ppm Cu(II) as copper sulphate. Temperature was 27·0 ± 2° C and oxygen content was 95 ± 10% saturation. The medium was changed once every two days. The pH was maintained at 6·5 ± 0·1. The mortality was noted every twenty-four hours. After a period of 20 days, six animals from each vessel were analysed for the total copper content of the whole soft tissues (*vide infra*).

In the second series of experiments the animals were treated similarly. At definite intervals (as shown in Figs. 3–5) samples of five animals each were dissected into different component organs, *viz.,* adductor muscle, gills, mantle and foot.

The various tissue samples were dried to constant weight at 80° C under vacuum. The dry tissue was homogenised into a fine powder, and ashed at 450–500° C. The ash was extracted with 1 : 1 HCl and the copper content of the sample was determined spectrophotometrically by the IUPAC carbamate method[17].

In all experiments, the animals were fed on the blue-green algae, *Synnechocystis* grown in a metal free medium; 5 ml of the suspension containing *ca.* 30,000 cells/ml were added to each tank, once every 24 hours. Blanks were run concurrently. Analysis on the composition of the medium used was also carried out. All chemicals used were of analytical reagent grade.

RESULTS AND DISCUSSION

Results are presented in Table I and Figs 1 to 5. Table I gives the data on the water quality. The important reactions which reduce the free metal-ion concentration in aquatic medium are[18,19]:

$$2\ Cu^{2+} + 2H_2O \rightleftharpoons Cu(OH)_2^{2+} + 2H^+ \quad (1)$$

and

$$Cu^{2+} + CO_3^{2-} \rightleftharpoons CuCO_3. \quad (2)$$

TABLE I

Water characteristics

S‰ = 10·5

Na	—	3800 ppm
K	—	150 ppm
Ca	—	560 ppm
Mg	—	242 ppm
Fe	—	1·1 μg at.l^{-1}
Cu	—	Nil
Cl$^-$	—	5820 ppm
SO_4^2	—	820 ppm
$NO_2 - N$	—	3·29 μg at. l^{-1}
$NO_3 - N$	—	4·16 μg at. l^{-1}
$PO_4 - P$	—	Traces
$SiO_3 - Si$	—	61·5 μg at. l^{-1}
pH	—	6·5 ± 0·1
Temperature	—	27 ± 2° C

Adsorption of cupric ions also decreases the amount of copper in solution. All these processes are, however, significant only at higher pH values.

Figure 1 gives % mortality *versus* time (in number of days) of exposure to Cu(II) salt solutions. The mortality observed in the initial stage stopped after a couple of days, the animals developing some resistance. The animals were found to survive even upto 2 months in an unbuffered medium of 10 ppm Cu(II). Earlier experiments on the effect of copper (II) on the black clam, *Villorita cyprinoides* var. *cochinensis* have shown that a concentration above 0·75 ppm is toxic and an LC_{50} value for a 10-day period was found to be 2 ppm[15].

The determination of copper content of the whole soft tissues of the clam reveals that rate of uptake is linearly dependent on the Cu(II) concentration of the medium. The least square plot of the rate of uptake (along with the actual values obtained) against concentration of Cu(II) is given in Fig. 2.

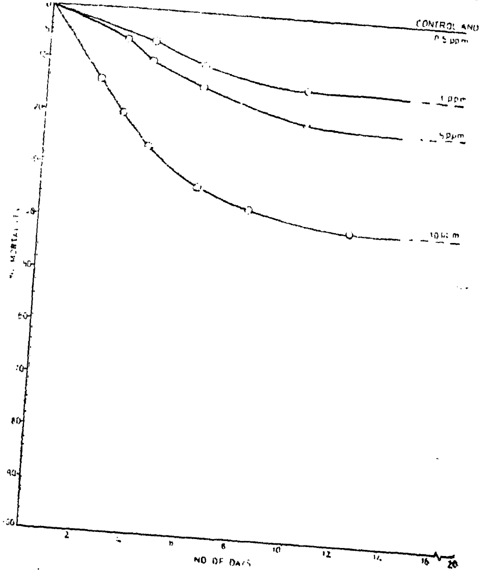


FIG. 1. Mortality (%) of *M. casta* exposed to various concentrations of Cu(II) solutions.

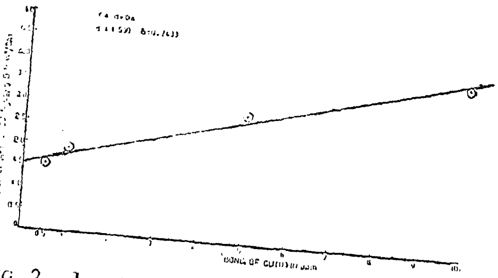
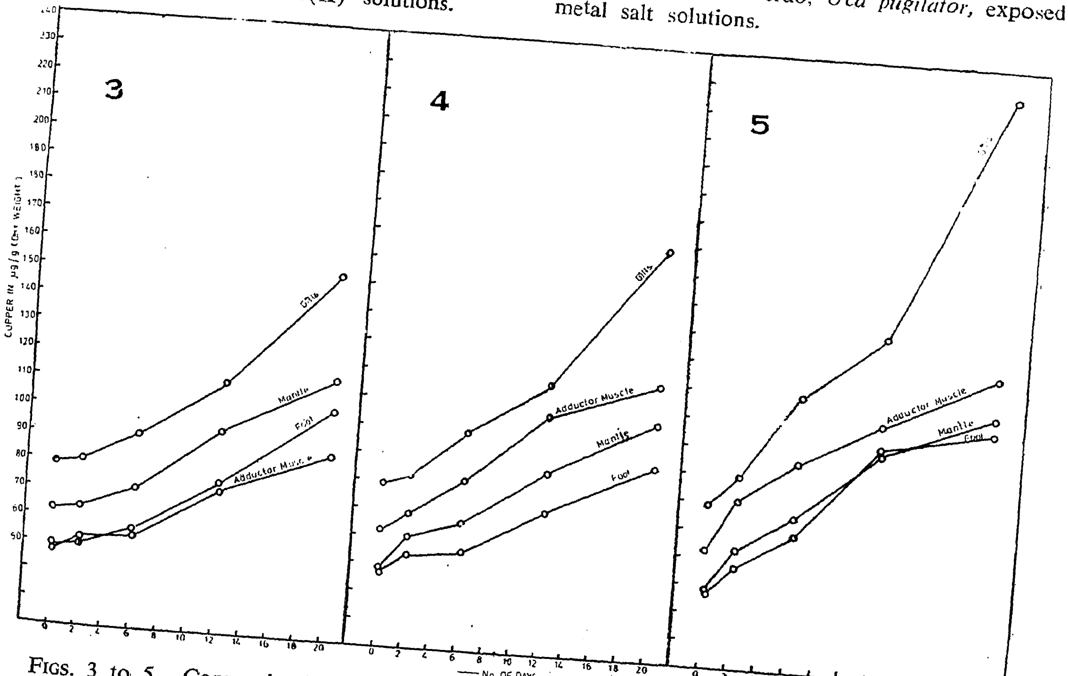


FIG. 2. Least square plot of copper uptake from aqueous solutions by *M. casta* as a function of Cu(II) concentration.

The results of analysis for the copper content of various organs (second experimental series) are presented in Figs. 3–5. In all components studied the copper content increased, but rates of uptake were different. Gills displayed by far the largest rate of uptake, the other organs showed a more or less equal rate of uptake. It may be mentioned here that a similar observation was made by Brookes and Rumsby[11] in the case of bivalves from New Zealand; Vernberg and Vernberg[20] have also observed a greater accumulation of metal in the gills of the Fiddler crab, *Uca pugilator*, exposed to metal salt solutions.



FIGS. 3 to 5. Copper in tissues of *M. casta* after exposure to various copper (II) concentra- ions in 10‰ sea water at 27 ± 2° C for varying lengths of time : Fig. 3—1 ppm ; Fig. 4—5 ppm and ig. 5—10 ppm.

It was observed on dissecting that the gills of all the clams maintained in 5 ppm and 10 ppm solutions had some deterioration; a change of colour from grey to yellow was observed. This may be due to the complexation of the copper in the gill. One of the suggested pathways[13] of heavy metal accumulation is by coordination through organic molecules, presumably, using the –NH and/or –SH groupings of the protein molecules. However, nothing much is known at present about the nature or composition of such complexes. The comparatively high amount of copper present in the gills may be because of the filter feeding nature of the animals. Other workers have also made similar observations.

The present study indicates that *Meretrix casta* could be successfully used for monitoring the heavy metal pollution of the aquatic environment.

ACKNOWLEDGEMENT

The authors thank Professor Dr. C. V. Kurian, Head of the Department, for the facilities and his keen interest in the work. They are also thankful to the University Grants Commission, Government of India, New Delhi, for financial assistance.

1. Calabrese, A., Collier, R. S., Nelson, D. A. and Mac Innes, J. R., *Mar. Biol.*, 1973, 18, 162.
2. John W. Arthur and Edward N. Leonard, *J. Fish. Res. Bd. Can.*, 1970, 27, 1277.
3. Kennth E. Biessinger, and Glem, M. Christensen, *Ibid.*, 1972, 29, 1691.
4. Arne Jensen, Britt Rystad, and Siguard Melsom, *J. Expt. Mar. Biol. and Ecol.*, 1974, 15, 145.
5. Davis, P. H., Goettl, J. P. J., Sinely, J. R. and Smith, N. F., *Water Res.*, 1976, 10, 199.
6. Brungs, W. A., Geckler, J. R. and Gast, M., *Ibid.*, 1976, 10, 37.
7. Thurberg, F. P., Dawson, M. A. and Collier, R. S., *Mar. Biol.*, 1973, 23, 171.
8. Cunningham, P. A. and Tripp, M. R., *Ibid.*, 1973, 20, 14.
9. Phillips, D. J. H., *Ibid.*, 1976, 38, 59.
10. Skidmore, J. F., *Quart. Rev. Biol.*, 1964, **30**, 227.
11. Robert R. Brookes, and Martin G. Rumsby, *Limnol. and Oceanogr.*, 1965, 10, 521.
12. Eustace, I. J., *Aust. J. Mar. and Fresh Wat. Res.*, 1974, 25, 209.
13. Benjamin H. Pringle, Dale E. Hissang, Edward L. Katz and Stefan T. Mulacoka, *Journal of the Sanitary Engineering Division, Proceedings of the American Society of Civil Engineers*, 1968, 94, 455.
14. Schulz–Baldes, M., *Mar. Biol.*, 1974, 25, 177.
15. Lakshmanan, P. T. and Krishnan Nambisan, P. N., *Indian J. of Mar. Sci.*, 1977 (In press).
16. Goldberg, E. D., In: *Chemical Oceanography*, ed., by Riley, J. P. and Skirrow, G., Academic Press, New York, 2nd ed., 1975, 3, 62.
17. Association of Official Analytical Chemists, *Official Methods of Analysis*, 11th Edn., McGraw-Hill, New York, 1970, p. 404.
18. Sylva, R. N., *Water Res.*, 1976, 10, 789.
19. Alberto Zirino and Sachio Yamamoto, *Limnol. Oceanogr.*, 1972, 17, 661.
20. Winona B. Vernberg, and John Vernberg, *Fishery Bulletin*, 1972, 70, 415.

USE REMOTE SENSING OF ENVIRONMENT*

THE cataloguing of the natural resources of a country is essential for its ecnomic growth. To do this by conventional means is an arduous and time-consuming task. Geologists have to travel through the length and breadth of the land exploring for minerals and ores; surveyors have to go to the most inhospitable places with their theodolites preparing maps; forest rangers have to wade through dense vegetation assessing the forest wealth; and agronomists have literally to move from field to field studying the conditions of the soil and crops and estimating the yield. All this will change even in developing countries when the science of remote sensing gets perfected and establishes itself as a new mode for natural resource survey.

Remote sensing is based on the simple concept that each feature of a terrain emits or reflects electromagnetic energy at specific and distinguishable wavelengths. The spectrum may vary from the shortest wavelength corresponding to gamma rays, going up the scale through the ultraviolet, the visible, the near infrared, the thermal infrared reaching microwave regions. Remote sensing can also be done by the precise measurement of gravitational and magnetic fields.

The cameras or devices that sense the electromagnetic spectrum can be mounted on different 'platforms'. These could be kites, balloons, aeroplanes and satellites; they are stabilised against roll, pitch, yaw and insulated against vibrations.

The optical camera was invented more than a hundred years back. However if it is to be used for aerial photography the aberration of the lens must be a minimum and these photographs can be interpreted only if they are sharp and undistorted. Very good aberration-free lenses have now been designed and roll films having good dimensional stability are being produced. In most aerial cameras, rollers guide the film surface of a locating plate. During an exposure suction is created which holds the film against this flat plate avoiding any possible distortion. Usually, both black and white and colour films, sensitive to the visible and near infrared, are used, thus increasing the information content of the photographs.

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* *Remote Sensing of Environment*. Edited by Joseph Lintz (Jr.) and David S. Simonett. (Addison Wesley Publishing Co., USA), 1976. Pp. xix + 694. Price $ 27.50.

The panoramic camera makes it possible to photograph a large area in a single exposure. The lens moves from side to side or in other words "pans" as the aircraft advances and the image is continuously recorded on a film held in the form of an arc of a circle. To minimise the aberration of the lens, a narrow angular field is provided by a narrow slit near the focal plane of the camera. This produces extremely sharp images.

The "multiband camera" with nine lenses, each having a carefully chesen combination of filter and film can record simultaneously nine photographs of the same terrain in different wavelength regions, ranging from the ultraviolet to the near infrared ($0 \cdot 4\,\mu$ to $0 \cdot 9\,\mu$).

One of the most important regions of the spectrum is the thermal infrared region which is quite insensitive to photographic film. Here one has to shield the sensor from the thermal radiations coming from the camera, equipment, etc. A rotating mirror directs the infrared energy emanating from a point on the terrain to a pinhead sized solid state detector which can be cooled by liquid nitrogen or liquid helium. The electrical signal generated at the detector is used to produce a visible picture on a cathode ray tube. By using proper detectors these "optical mechanical scanners" can provide multiband imagery from the near ultraviolet to the thermal infrared.

Another revolutionary development is the side looking airborne radar (SLAR) which brings to remote sensing an all-weather, round-the-clock use. The basic principle of this system is simple but its practical achievement involves extremely complimated technology. The transmitting antenna in the plane sends a short pulse of microwave energy out of one side of the plane. The pulse strikes the ground and the reflection is collected by a sensitive antenna system. There is a time delay between the incident pulse and the reflected one which can accurately be measured, the delay being greater for that part of the terrain that is farther away from the plane. Another changing parameter is the frequency of the reflected signal which changes (due to Doppler effect) according to whether the plane is approaching a point or receding from it. SLAR uses these two discriminatory effects, *viz.*, the time delay and the frequency change to obtain a synthetic aperture which increases the spatial resolution enormously. The antenna receives the reflections directly opposite to the aircraft, *i.e.*, the part exhibiting no Doppler effect. The radar images are again transformed into photographic image.

The analysis of the remote sensing 'pictures' depends on the spectral response patterns, or if one uses the jargon 'multiband tone signature' given by each feature. It is therefore essential to study known objects on test sites and record their unique response patterns to various wavelengths. As the number of spectral bands increases, the recognition pattern of each 'natural resource' on environmental features becomes more certain.

The analyst is finally confronted with a large number of black and white images each corresponding to a spectral band each with distinctive tone values. Such a situation can be very confusing and many techniques have been evolved for interpretation. One method to deal with this problem is to reconstitute the multispectral black and white images into a single composite colour picture using colour filters. By changing the colour filters, the colour contrast can be varied, one effective combination providing the best interpretability for one kind of feature of interest. The second method is to use photo-electric sensors to scan all the black and white images so that for each spot scanned, with its built-in memory system, the sensor can automatically determine the tone signature which will result in a tape print-out indicating the 'objects' and identification of the conditions encountered. The third method, which is the most sophisticated and desirable one, uses a magnetic tape with a memory and microprocessing capability making it possible to have an inventory of all relevant identification only a few seconds after the remote sensors have flown over the area of interest. The information thus obtained can be telemetered to the earth. It need not be mentioned that these processes require the use of very sophisticated electronics and computers and software.

The capabilities of remote sensing techniques *via* satellite or even an aeroplane are myriad. In agriculture it can give information on the type of crops growing in each field, and the possible yield. The plant stem rust in wheat, the blight in potato or the coconut disease are easily recognisable by the change in the reflectance of the plant in the near infrared region. Unhealthy individual plants can be identified from satellites. Since plant diseases can completely destroy the annual production targets, the importance of early detection of these on the economy of a country is of the greatest significance.

Water resources can be sensed and managed by remote sensing. One can locate and even estimate the volume of the surface and sub-surface flow in water sheds. The area and depth of snow can be determined fairly accurately permitting the regulation of the impounding and release of waters in reservoirs.

Geographers can analyse land use patterns and study the effect of climate on plant life. Engineers planning large construction projects can obtain data on topography, rocks, minerals, soils and conditions of drainage. Minerals and petroleum can be detected. Soil scientists can not only record the physical and chemical characteristics of the soils but correlate these with the geological features. The forester can detect fires and estimate state of health and wealth of a forest.

Ocean currents can be mapped, distribution of marine organism and marine animals can be seen, water pollution can be determined. Daily and seasonal changes and the state of the sea can all be monitored. These are only a few of the benefits that can accrue from remote sensing.

But even in this brief popular account one can see that remote sensing is a very complex field. In the book under review the editors have collected articles written by experts covering practically all the aspects of remote sensing using electromagnetic spectrum. The first part deals with the physics of remote sensing together with the problems of the effect of atmospheric absorption on data accumulation and how this can be overcome by judicious data processing. The philosophical problem of remote sensing is also dealt with in one chapter.

The second part deals with instrumentation of remote sensing—the photographic, the non-photographic, the passive and the active microwave systems, sensors for space aircraft and geophysical airborne systems. Part three deals with data processing, qualitative and quantitative image analysis. The fourth and the final part deals in detail with many applications of remote sensing —uses in agriculture (crop identification, crop diseases, insect infestation), in forestry (tree identification, study of diseases, insects and pollution in forests), in geography (scale and resolution, dimensional change detection of urban characteristics, land use studies, thematic mapping); in geology and geophysical prospecting, in water resource survey (hydrology, contaminant dispersion in water bodies, ground water systems); in oceanography (physical, biological, geological, chemical oceanograph, maritime weather, fisheries, coastal engineering and hydrology, sea ice). It is a comprehensive book. Some of the chapters are lucidly written while others (by the very nature of the contents) make difficult reading. Reviewer feels that this book must be bought by all the libraries in India to enable our scientists and students to become aware of the importance of this field, so necessary for our economic growth.

S. R.

SECOND ALL INDIA SYMPOSIUM OF DEVELOPMENTAL BIOLOGY

The Second All India Symposium of Developmental Biology will be held from 5th to 7th November 1977 at the Department of Zoology, Poona University. The Symposium will have six sessions: Differentiation and Morphogenesis, Cell and Tissue Culture Developmental Genetics and Molecular Biology, Regeneration, Teratogenesis, Developmental Approach for Insect Control. For further details please contact: Professor Leela Mulherkar, Department of Zoology, Poona University, Poona 411 007.

SYMPOSIUM ON STUDY AND MANAGEMENT OF WATER RESOURCES IN ARID AND SEMI-ARID REGIONS

A symposium on the above subject will be held in March 1978 at Physical Research Laboratory, Ahmedabad. The following subjects will receive major attention: (1) Use of Tracers, (2) Use of Simulation Models, (3) Soil-Water-Plant Relationship, (4) Use of Remote Sensing and Aerial Photographs, (5) Water Resource Management. Any other topic for discussion at this symposium may be suggested.

Written abstracts (about 3 pages) must reach PRL by 15th December 1977. Arrangements are being made to publish a separate volume containing symposium proceedings including discussions. Last date for submission of completed paper is 1st February 1978.

Further details can be had from Dr. S. K. Gupta, Convener, Room No. 258, Physical Research Laboratory, Ahmedabad 380 009.

LETTERS TO THE EDITOR

SPECTROPHOTOMETRIC DETERMINATION OF OSMIUM WITH MEPAZINE HYDROCHLORIDE

Mepazine hydrochloride, MH, 10-[(1-methyl-3-piperidyl) methyl] phenothiazine hydrochloride is recommended as a new reagent for the spectrophotometric determination of osmium(VIII). The method involves the formation of a red coloured species which is believed to be a radical cation in 1·0–3·0 N HCl. For full development of colour intensity a ten-fold molar excess of the reagent is required. The red species exhibits maximum absorbance at 512–516 nm. The reagent and the metal do not absorb at this wavelength. The maximum colour development takes place after 8 minutes and the absorbance readings remain constant for one hour at 10–50° C. No change in absorbance is observed when the order of addition of reactants was varied. Beer's law is valid over the concentration range 0·16–6·4 ppm and the optimum range as evaluated by Ringbom's method[1,2] is 1·0–6·2 ppm. According to Sandell's expression, the sensitivity of the reaction is 0·018 μg/cm^2 and the molar absorptivity is $1 \cdot 05 \times 10^4$ litre mol^{-1} cm^{-1} at 515 nm.

Sample solutions containing 4 μg/ml of osmium prepared by the standard procedure gave a relative error of $\pm 0 \cdot 7$% and standard deviation of 0·002. The following amounts (μg/ml) of diverse ions are found to give less than ± 2% error in the determination of 4 μg/ml of osimum(VIII): rhodium(III) 15, iridium(III) 14, platinum(IV) 2, ruthenium(III) 0·6, palladium(II) 0·6, iron(III) 2, cobalt(II) 72, nickel(II) 660, copper(II) 70, fluoride 1460, chloride 4500, bromide 1900, iodide 3, nitrate 1575, phosphate 1420, sulphate 6000, acetate 7340, oxalate 920 and EDTA 5800.

Synthetic mixtures corresponding to osmiridium alloy were prepared and the osmium content was determined following the standard pocedure. The results are given in Table I. The proposed method offers the advantages of simplicity, rapidity and good sensitivity without the need for heating or extraction.

Standard procedure.—To an aliquot of the sample solution containing 4 to 160 μg of osmium(VIII) (prepared from osmium tetroxide in 0·2 M sodium hydroxide and standardized iodimetrically[3]) were added 8·5 ml of 6N HCl and 2 ml of 0·2% aqueous MH solution and the volume was made up to 25 ml by adding doubly distilled water. The solution was mixed well and the absorbance was measured at 515 nm after standing 8 minutes against a reagent blank prepared similarly.

Table I

Determination of osmium in synthetic mixtures corresponding to osmiridium alloy

Os(VIII) taken, ppm	Ir(III) added, ppm	Os(VIII) found*, ppm
1·20	0·3	1·20
2·40	0·6	2·39
3·60	0·9	3·59
4·80	1·2	4·79
6·00	1·5	5·99

* The amount of osmium (VIII) found is based on ten determinations.

M/s. Byk Gulden Lomberg Chemische Fabrik, GmbH, Konstanz, West Germany, are thanked for supplying pure mepazine hydrochloride.

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October 11, 1976.

1. Ringbom, A., *Anal. Chem.*, 1938, **115**, 332.
2. Ayres, G. H., *Ibid.*, 1949, **21**, 652.
3. Crowell, W. R. and Kirschman, *J. Amer. Chem. Soc.*, 1929, **51**, 175.

THERMODYNAMIC FUNCTIONS OF SOME BIVALENT METAL ION CHELATES OF DL-α-AMINOBUTYRIC ACID

Introduction

In previous publications[1-3] from this laboratory, the stability constants of some amino-acid metal chelates, including those formed with α-aminobutyric acid have been reported. It is recognised that for a proper understanding of chelation reactions, a knowledge of the thermodynamic functions is imperative. Gergely *et al.*[4] have earlier reported thermodynamic functions of some common bivalent transition metal chelates of α-aminobutyric acid. In the present paper we report the thermodynamic functions ($\triangle$G, $\triangle$H and $\triangle$S) of Be(II), Cd(II) and Hg(II) chelates of DL-α-aminobutyric acid. Their formation has been potentiometrically studied by Irving and Rossotti's[5] method.

Experimental

Three mixtures (acid), (acid + ligand) and (acid + ligand + metal ion) were potentiometrically titrated separately with 0·1 M NaOH solution in a double walled titration vessel with a sealed on water jacket at a fixed temperature. Total volume was kept at 50 ml each time and the ionic strength of 0·1 M was maintained by adding $NaClO_4$ solution. Purified nitrogen was bubbled both before and during the titration to exclude CO_2. pH measurements were done by a Leeds–Northrup pH-meter using glass electrode. From the shifts in the pH titration curves, the values of $\bar{n}_A$, $\bar{n}$ and *p*L were calculated by Irving and Rossotti's[5] method.

Results and Discussion

The protonation constants as well as metal-ligand stability constants, as computed by correction term and successive approximation methods, at different temperatures, are given in Table I.

The enthalpy change (ΔH) has been determined by temperature coefficient method by the equation :

$$\Delta H = 2{\cdot}303\ RT_1T_2 (\log K_2 - \log K_1)/(T_2 - T_1)$$

and the free energy and entropy changes were calculated as :

$$\Delta G = -\ 2{\cdot}303\ RT\ \log K$$

and

$$\Delta G = \Delta H - T\Delta S.$$

The results are reported in Table II.

It is observed that the stability constants show a regular decrease with the increase in temperature.

A perusal of thermodynamic functions shows that Be(II) chelates have a very favourable enthalpy change for the formation of first chelated complex whereas Hg(II) chelates are more entropy stabilized. The entropy change for the second step in Be(II) system is rather anomalous.

TABLE I

Stability constants of Be, Cd and Hg chelates of DL-α-aminobutyric acid at different temperatures
(μ = 0·1 M $NaClO_4$).

Metal ion		Stability constants				
		20° C	25° C	30° C	35° C	40° C
Be(II)	Log K_1H	9·62±0·02	9·49±0·02	9·35±0·03	9·22±0·02	9·07±0·03
	Log K_2H	2·35±0·01	2·30±0·02	2·26±0·02	2·23±0·01	2·20±0·01
	Log K_1	6·90±0·02	6·80±0·03	6·71±0·02	6·61±0·02	6·52±0·03
	Log K_2	5·95±0·02	5·90±0·01	5·86±0·01	5·82±0·01	5·77±0·02
	Log β_2	12·85±0·04	12·70±0·04	12·57±0·03	12·43±0·03	12·29±0·05
Cd(II)	Log K_1	3·49±0·01	3·46±0·02	3·42±0·01	3·39±0·01	3·35±0·02
	Log K_2	3·44±0·01	3·40±0·02	3·35±0·02	3·31±0·02	3·27±0·02
	Log β_2	6·93±0·02	6·86±0·04	6·77±0·03	6·70±0·03	6·62±0·04
Hg(II)	Log K_1	6·61±0·03	6·55±0·02	6·50±0·01	6·46±0·02	6·41±0·02
	Log K_2	5·51±0·02	5·47±0·02	5·44±0·01	5·41±0·01	5·38±0·01
	Log β_2	12·12±0·05	12·02±0·04	11·94±0·02	11·87±0·03	11·79±0·03

TABLE II

Thermodynamic functions of Be, Cd and Hg chelates of DL-α-aminobutyric acid
(Temp. 20° C, μ = 0·1 M $NaClO_4$)

Metal ion	$-\Delta G_1$ Kcal/mole	$-\Delta G_2$ Kcal/mole	$-\Delta H_1$ Kcal/mole	$-\Delta H_2$ Kcal/mole	ΔS_1 e.u.	ΔS_2 e.u.
Be(II)	9·28±0·04	8·05±0·01	7·7±0·1	3·7±0·1	5±1	15±1
Cd(II)	4·72±0·03	4·64±0·03	3·1±0·1	3·4±0·1	5±1	5±1
Hg(II)	8·94±0·03	7·46±0·03	4·5±0·1	2·8±0·1	15±1	16±1

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1. Singh, M. K. and Srivastava, M. N., *J. Inorg. Nucl. Chem.*, 1972, 34, 567 ; 2067 ; 2081 ; *Talanta*, 1972, 19, 699.
2. Tewari, R. C. and Srivastava, M. N., *J. Inorg. Nucl. Chem.*, 1973, 35, 2441, 3044 ; *Talanta*. 1973, 20, 133, 360 ; *Indian J. Chem.*, 1973, 11, 700, 1196 ; *Acta Chimica* (Hungary), 1974, 83, 259.
3. Srivastava, J. P. N. and Srivastava, M. N., *Indian J. Chem.*, 1976, 14 (In press) ; *Talanta*, 1977, 24, (In press) *Vij. Pari. Anu. Patrika*, 1976, 19, 117.
4. Gergely, A., Mojes, J. and Kassaibazsa, Zs., *J. Inorg. Nucl. Chem.*, 1972, 34, 1277.
5. Irving, H. and Rossotti, H. S., *J. Chem. Soc.*, 1953, p. 3397 ; *Ibid.*, 1954, p. 2904.

SYNTHESIS OF KANZAKIFLAVONE-1 AND KANZAKIFLAVONE-2

MUNEHISA ARISAWA *et al.*[1,2] have recently isolated kanzakiflavone–1 (I) and kanzakiflavone–2 (II) from *Iris unguicularis* POIR and assigned to them, the structures 5, 8-dihydroxy-4′-methoxy-6, 7-methylenedioxyflavone and 5, 4′-dihydroxy-6,7-methylenedioxyflavone respectively on the basis of spectral and chemical studies. In order to confirm the above structures, we have synthesised I and II which are identical with the natural compounds in all respects.

For the synthesis of kanzakiflavone–2 (II), a mixture of scutellarein[3] (III) (500 mg), methylene iodide (0·4 ml), dry acetone (100 ml) and ignited K_2CO_3 (2 g) was refluxed for 18 hrs. The mixture was filtered and the solvent distilled. Water was added to the residue and the yellow solid which separated was filtered. It was purified by passing over a column of silica gel and crystallised from alcohol as yellow prisms (200 mg), m.p. 311–312° (lit.[2] m.p. 310°).

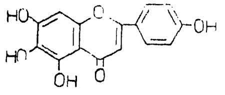
UV: λ_{max}^{MeOH} 284, 334 nm (log ϵ 4·12, 4·32)

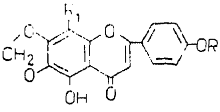
λ_{max}^{MeOH} + $AlCl_3$: 290 (sh), 295, 360 nm (log ϵ 4·10, 4·19, 4·32).

IR: ν_{max}^{nujol} 3300, 1680, 1030, 930 cm^{-1}

NMR ($CDCl_3$, δ) 7·65 (d, J = 9·0 Hz, 2H at $C_{2'}$ and $C_{6'}$ positions),
6·82 (*d*, *J* = 9·0 Hz, 2H at $C_{3'}$ and $C_{5'}$ positions),
6·50 (s, 1H, C_8H), 6·30 (s, 1H, C_3H), 6·00 (s, 2H, O–CH_2–O).

The spectral data of this compound were found to be in full agreement with the data given in literature[2] for (II).


III


I R₁ = OH , R₂ = CH₃
II R₁ = R₂ = H
IV R₁ = H , R₂ = CH₃

For the synthesis of Kanzakiflavone–1 (I), (II) (300 mg) was partially methylated with dimethyl sulphate (0·14 g), dry acetone (5 ml) and K_2CO_3 (2·0 g) by refluxing for 6 hrs. On working up in the usual way, it gave the methyl ether (IV) which crystallised from CH_3OH as pale yellow needles (200 mg), m.p. 250–252°.

UV: λ_{max}^{MeOH} 280, 330 nm (log ϵ 4·10, 4·30)

IR: ν_{max}^{nujol} 1680 cm^{-1} (carbonyl)

NMR: ($CDCl_3$, δ) 7·65 (d, J = 9·0 Hz, 2H at $C_{2'}$ and $C_{6'}$ positions),
6·82 (d, J = 9·0 Hz, 2H at $C_{3'}$ and $C_{5'}$ positions),
6·50 (s, 1H, C_8H), 6·30 (s, 1H, C_3H), 6·00 (s, 2H, –O–CH_2–O–), 3·80 (s, 3H, OCH_3).

The methyl ether (IV) (200 mg) was dissolved in aqueous KOH (10 ml, 5%) and pyridine (5 ml) added. The solution was stirred at 15–20° and $K_2S_2O_8$ solution (500 mg in 40 ml of H_2O) added dropwise during the course of four hours. The solution was left overnight and acidified with dil. HCl to Congo red. The precipitated unreacted compound was filtered and the filtrate extracted with ethyl acetate to ensure complete removal of the unreacted compound. The aqueous layer was treated with conc. HCl (6 ml) and Na_2SO_3 (100 mg) and heated on water bath (70°) for 30 min. when a brown mass separated. It was filtered and crystallised from pyridine-methanol as yellow needles (60 mg), m.p. 291–293° (lit.[1] m.p. 291–293°).

UV: λ_{max}^{MeOH} 280, 345 nm (log ϵ 4·11, 4·30)

λ_{max}^{MeOH} + $AlCl_3$ 295, 370 nm (log ϵ 4·10, 4·28)

IR: ν_{max}^{nujol} 3340, 1680, 1030, 930, 770 cm^{-1}

NMR: ($CDCl_3$, δ) 7·80 (d, J = 9·0 Hz, 2H at $C_{2'}$ and $C_{6'}$ positions),
6·80 (d, J = 9·0 Hz, 2H at $C_{3'}$ and $C_{5'}$ positions),
6·42 (s, 1H, C_3H), 5·96 (s, 2H, O–CH_2–O), 3·82 (s, 3*H*, OCH_3).

The spectral data of this compound were found to be in full agreement with the literature[1] data of kanzakiflavone 1 (I).

Our thanks are due to Professor M. Arisawa, Faculty of Pharmaceutical Science, Toyama University, for the supply of the natural samples of kanzakiflavone-1 and kanzakiflavone-2.

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1. Munehisa Arisawa and Naokata Morita, *Chem. Pharm. Bull.*, 1976, **24** (4), 815.
2. —, Haruhisa Kizu and Naokata Morita, *Ibid.* 1976, **24** (7), 1609.
3. Shashtri, V. D. N. and Seshadri, T. R., *Proc. Indian Acad. Sci.*, 1946, **23 A**, 262.

WILLGERODT REACTION MODIFIED: APPLIED TO CHALCONES

IN Willgerodt reaction[1] aryl alkyl ketone is treated with an aqueous solution of yellow ammonium polysulphide. In 'Kindler Variation' of Willgerodt reaction, the ketone is heated with an equal amount of sulphur and amine. The thioamide intermediate, on acid or alkaline hydrolysis gives the acid *e.g.* acetophenone gives phenyl acetic acid.

$$C_6H_5COCH_3 + S + \text{morpholine} \rightarrow C_6H_5CH_2\,(S)\,NC_4H_8O \rightarrow C_6H_5CH_2COOH.$$

It was thought interesting to apply this modified W.R. to chalcone and substituted chalcones. The amine used in this experiment was morpholine.

In comparison to the reaction of acetophenone in W.R., the chalcone should undergo a reaction in the following manner:

$$C_6H_5COCH = CHC_6H_5 \rightarrow C_6H_5CH = C\,(C_6H_5).COOH.$$

However, chalcone gave an unsaturated acid but not the expected α-phenyl cinnamic acid as shown above. The acid on identification was found to be cinnamic acid.

$$C_6H_5CO.CH = CHC_6H_5 \rightarrow C_6H_5CH = CHCOOH$$

When 2′-hydroxy-5′-methyl-4-methoxy chalcone was subjected to the modified W.R. the acid obtained was identified as *p*-methoxy cinnamic acid. Again 2′-hydroxy chalcone gave cinnamic acid.

These results definitely suggest that with α, β-unsaturated ketone the W.R. gives unsaturated acids by the break in C–C linkage at ketone group. The second product in the above reaction was found to be *p*-cresol and phenol respectively.

Survey of the literature does not give any such reaction in which the break is at the ketone group in chalcone derivatives. Generally different reactions of oxidation type break the molecule of chalcone at the double bond.

The chalcone on acid or alkali hydrolysis gives back ketone and benzoic acid. No break at the =CO group giving α, β-unsaturated acid is known. Looking from this point of view the reaction under investigation is a novel type reported here for the first time.

This reaction has an immense potentiality because it can be used for the proper identification of naturally occurring flavonoid series. Another advantage in degradative study of chalcone by this modified W.R. is that the methoxy groups present in either ring remain intact. In the case of alkali degradation methods, the methoxy groups are usually lost.

Experimental.—Chalcone (3 g) an equal quantity of sulphur powder (3 g) and morpholine (10 ml) (redistilled at 128° C) were taken in a round bottom flask (250 ml). The reaction mixture was refluxed for 5 hrs and kept overnight. Crystals separated were filtered, washed with water till the smell of morpholine was removed and crystallised from methanol. The yellow crystalline thiomorpholide of chalcone was obtained. The thiomorpholide of chalcone was hydrolysed with dil. HCl. A white solid was obtained. It was crystallised from hot water to get cinnamic acid (1·7 g) or substituted acid. The filtrate of the thiomorpholide was subjected to isolation of cresol, yield = 1 g.

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Amravati, *June* 16, 1977.

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1. Conrad Willgerodt, *Ber.*, 1887, 20, 2467. (Roger Adams, *Organic Reactions*, Vol. III, Wiley, 1949, p. 83).

APPLICATION OF HAMMETT EQUATION TO GUANYL PYRAZOLE NITRATES

Introduction

IN our earlier communication[1] we had reported our results on the effect of substituents on the half wave potential of 4-phenylazo-N'-guanyl-3, 5-dimethyl pyrazole nitrate qualitatively. As in all these compounds the value of $dE_{\frac{1}{2}}/dpH$ and diffusion current constant (I) for the reduction of azo group and the value of *pK* for the reduction of azomethine group were found almost constant, the conditions of applying Hammett equation are fulfilled[2] and thus it was considered worthwhile to correlate the half wave potential of these compounds with Hammett substituent constant (σ) in order to express the effect of substituents quantitatively.

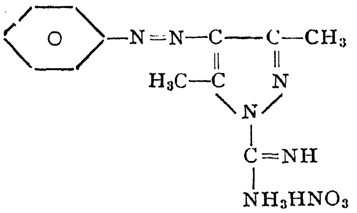
Results and Discussions

For the study of substituent effects quantitatively, the waves in a Britton Robinson buffer of pH 3·8 (at which best waves were obtained) were measured and the $E_{1/2}$ compared (Table I). The values of Hammett substituent constant (σ) were used from 0-benzoic acids.[3,4]

Figure 1 (*a*) represents a linear plot of $-E_{1/2}$ *vs* σ for the reduction of the azo group. It is clear from this plot that except 3-CH_3, all other meta- and para-derivatives fit in the straight line and ortho-derivative, *viz.*, 2-Br, 2-Cl, 2-OCH_3, 2-CH_3, 2, 3 $(CH_3)_2$, 2, 4 $(CH_3)_2$ and 2, 6 $(CH_3)_2$ deviate significantly from the linear plot. The deviation of ortho derivatives can

TABLE I

Half-wave-potential of substitued guanyl pyrazole nitrates at pH 3·8 concentration 1 × 10⁻⁴ M.



where R are various substituents.

Sl. No.	R	$(-E_{1/2})_1$	I^{*a}	$(-E_{1/2})_2$	σ
1.	H	0·36	0·20	0·82	0·0
2.	2–Cl	0·29	·19	0·78	0·20
3.	3–Cl	0·31	·18	0·80	0·37
4.	4–Cl	0·32	·20	0·84	0·23
5.	2–CH_3	0·34	·20	0·83	–0·17
6.	3–CH_3	0·34	·20	0·88	–0·07
7.	4–CH_3	0·40	·18	0·86	–0·17
8.	2–COH_3	0·34	·19	0·84	–0·39
9.	3–OCH_3	0·34	·20	0·84	0·11
10.	4–OCH_3	0·40	·20	0·87	–0·27
11.	2–Br	0·30	·18	0·82	+0·21
12.	3–Br	0·30	·18	0·82	+0·39
13.	4–Br	0·32	·20	0·88	0·23
14.	2, 3–$(CH_3)_2$	0·34	·21	0·83	–0·24
15.	2, 4–$(CH_3)_2$	0·33	·19	0·84	–0·34
16.	2, 6–$(CH_3)_2$	0·33	·19	0·83	–0·34

a— micro amp./millimole/$mg^{2/3}S^{-1/2}$.

$(-E_{1/2})_1$ and $(-E_{1/2})_2$ are for the reduction of azo group and azo methine group respectively.

* For the reduction of azo group.

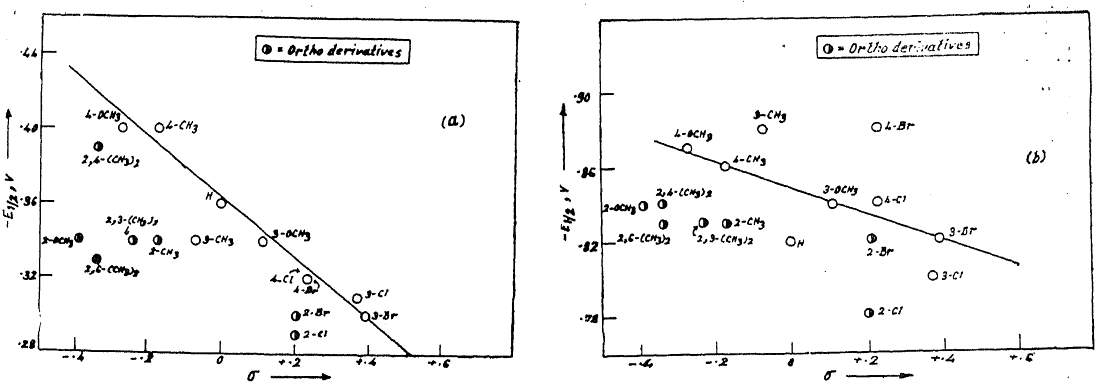


FIG. 1, Plots of—$E_{1/2}$ *vs* σ for the reduction of (*a*) azo group, (*b*) azo methine group in guanyl pyrazole nitrates.

be readily accounted for on the basis of ortho effects[5]. The value of specific reaction constant (ρ) for the reduction of this group was found to be 0·14 which is comparable with the values reported in the literature[4,6] for the similar systems. The positive value of ρ points to a nucleophilic mechanism of the electrode process (electron uptake as the potential determining step).

In disubstituted derivatives, it was found that 2;3 and 2; 6 disubstituted derivative do not show additive effect, whereas 2, 4-dimethyl derivative shows a reasonable additivity of 2 and 4 shifts. Zuman[2] has reported that the values of polarographic ortho shift can be obtained by the comparison of $E_{1/2}$ of ortho and para substituted compounds. Table I.

TABLE II

Polarographic ortho shift ($\triangle_0$) *for the reduction of azo group*

Sl. No.	R	Δ_0,V
1.	Chloro	0·030
2.	Bromo	0·020
3.	Methyl	0·060
4.	Methoxy	0·060

gives the values of polarographic ortho shift (Δ_0) for methoxy, methyl, chloro and bromo substitutents. It is clear from the positive value of Δ_0, that ortho substituted derivatives undergo reduction more easily in comparison to *p*-derivatives.

In the polarographic reduction of azomethine group, however, H and 4-Br substituents found to show deviation from the linear plot of $E_{1/2}$ *vs.* Hammett substituent constant (σ). The deviation of these substituents from the regression line can be explained. The substituents are remote from the reduction site (— C = NH) and hence the change in half wave potential was not systematic (Table I). The value of specific reaction constant was found to be 0·09 V, which is in good agreement with the values for similar systems.[6,7]

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---

1. Malik, W. U. and Goyal, R. N., *Talanta*, 1976, **23**, 705.
2. Zuman, P., *Substituents Effects in Organic Polarography*, Plenum Press, New York, 1967, p. 39.
3. Jaffe, H. H., *Chem. Rev.*, 1953, **53**, 191.
4. Hammett, L. P., *Physical Organic Chemistry*, McGraw-Hill, New York, 1940.
5. Zuman, P., *Coll. Czech. Chem. Commun.*, 1962, **27**, 648.
6. Kitajiv, P. Yu, Budnikov, G. K. and Arbuzov, A. E., *IZv. Akod. Hank USSR. Otd. Chem. Nank*, 1961, p. 1772.
7. Fleet, B. and Zuman, P., *Coll. Czech. Chem. Commun.*, 1966, **32**, 2066.

FLAVONOL GLYCOSIDES FROM THE LEAVES OF *RHUS PARVIFLORA*

Rhus parviflora Roxb. (family : Anacardiaceae), an indigenous medicinal plant[1], was examined earlier by Bhakuni *et al.*[2], who reported the presence of isorhamnetin-3-arabinoside and quercetin. Continuing our search for new myricetin glycosides[3] and in view of the earlier report of myricetin in *Rhus* species[4] we have examined the leaves of *R. parviflora* : our isolation of three flavonols (both free and as 3-O-rhamnosides) and two aromatic acids is given below.

The hot aqueous alcoholic concentrate of shade-dried leaves of *R. parviflora,* collected from Garhwal, U.P., was partitioned using petrol, ether and ethyl acetate. The flavonols and carboxylic acids of the ether layer separated by means of $NaHCO_3$ were purified by preparative TLC on silicic acid. The three flavonols were identified as myricetin, quercetin and kaempferol by their R_f, UV and IR spectra as well as PMR and MS of their acetates ; the identity of the compounds was also confirmed by co-TLC with authentic samples. The acids were found to be *p*-coumaric acid and caffeic acid by m.p., UV fluorescence and preparing their acetates ; co-TLC with authentic samples finally established their identity.

The flavonol glycosides from the EtOAc concentrate was separated first by preparative TLC (SiO_2, $CHCl_3 : MeOH = 1 : 1$), followed by preparative PC (Whatman No. 3 paper, water). The three purified components were subjected to various colour reactions, UV fluorescence, acid hydrolysis and characterisation of hydrolytic products. All of them were found to be 3-O-glycosides and the single sugar was rhamnose. The aglycones were identified as myricetin, quercetin and kaempferol. We could not detect any isorhamnetin in spite of detailed comparative study using authentic sample.

The identity of three glycosides as myricetin-3-O-rhamnoside (myricitrin ; major), quercetin-3-O-rhamnoside (quercitrin) and kaempferol-3-O-rhamnoside (afzelin) by their R_f, m.p., λ_{max} and ν_{max} was also confirmed by direct comparison with authentic samples isolated from *Madhuca indica*[5], *Soymide febrifuga*[6] and *Tilia cordata*[7] respectively.

Our finding of the occurrence of myricetin glycoside as the major flavonoid component in *R. parviflora* is quite justifiable from the point of view of the flavonoid pattern[4] of other *Rhus* species. *R. parviflora* appears to be a good source for myricitrin (yield, *ca.* 1%).

The presence of myricetin (5′-hydroxy quercetin ; hydroxylation) and kaempferol (3′-deoxy quercetin, reduction) in place of isorhamnetin (3′-methyl quercetin, O-methylation) reveals a minor deviation in the later stage of biosynthesis of flavonoids and may be influenced by ecological variation.

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and I.I.T., Madras, for the spectral data. J. P. K. thanks the Director, C.C.R.I.M.H., New Delhi, for encouragement.

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April 16, 1977.

---

1. Chopra, R. N., Nayar, S. L. and Chopra, I. C., *Glossary of Indian Medicinal Plants*, C.S.I.R., New Delhi, 1956.
2. Bhakuni, D. S., Satish, S., Shukla, Y. N. and Tandon, J. S., *Phytochem.*, 1971, 10, 2829.
3. Nair, A. G. R. and Subramanian, S. S., *Indian J. Chem.*, 1972, 10, 452.
4. Reznik, V. K. and Egger, K., *Z. Naturforsch.*, 1960, 15, 247.
5. Nair, A. G. R. and Subramanian, S. S., *Phytochem.*, 1972, 11, 3090.
6. — and —, *Indian J. Chem.*, 1975, 13, 527.
7. Horhammer, L., Stich, L. and Wagner, H., *Naturwissenschaften*, 1959, 46, 358.

NUCLEAR PHENOTYPE OF *SOLANUM INTEGRIFOLIUM*

Cytological information collected on the nuclear phenotypes (form and structure of chromosomes at pachytene) of parental genomes and the pairing behaviour of chromosomesin the F_1 heterozygotes have been profitably used in the past by different workers in understanding species relationships and evolutionary trends in related taxa. Among the spinous Solanums, many of which are of economic importance, only a few have been studied cytologically to reveal the structure of their pachytene chromosomes[1-3] and their inter-relationships remain uncertain[2,3]. Since furthering our knowledge in this direction is a prerequisite to their better utilization through cytogenetic manipulation, we have been studying the pachytene chromosomes of some members, and their hybrid derivatives. Our results on the chromosome complement of *Solanum integrifolium* Poir. ($2n = 24$) are reported in this note.

Following the customary techniques of fixing the flower buds in 1:3 acetic-alcohol and smearing in acetocarmine, observations were made on PMCs at pachytene. All the 12 bivalents could be traced from end to end in some nuclei and their morphological features ascertained from an analysis of the entire genome. The chromosomes have been identified individually as Ch. 1 to Ch. 12 on the basis of their lengths, and their diagnostic criteria established.

The salient features of the nuclear phenotype in *S. integrifolium* are:

The chromosomes are differentiated into hetero- and euchromatic regions to varying degrees on different chromosomes; the total length varies from about 35 microns for chromosome 1 to about 13 microns for chromosome 12; most of the chromosomes have their centromeres located submedianly with arm ratios varying around 1·4; there is a single nucleolus organising bivalent which is highly eccentric with an arm ratio of 6·3 (sub-terminal), and occupies the tenth position in the complement; the nucleolar body is located terminally on the short arm which is satellited. The diagnostic criteria that enable their identification are: (*a*) Total length; (*b*) Arm ratio, reflecting the position of the centromere, and more importantly (*c*) The extent to which the chromosomes are differentiated into hetero- and euchromatic regions in each of their arms (Fig. 1).

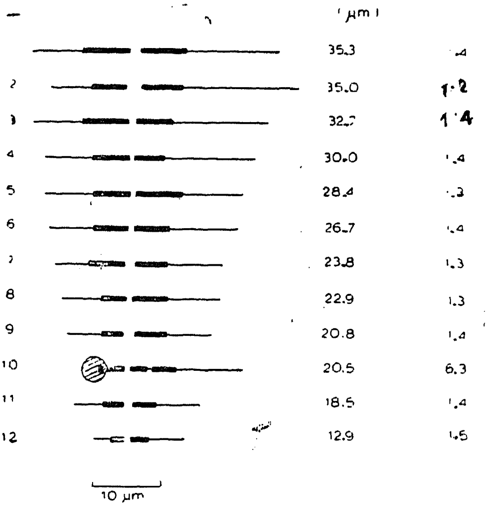

FIG. 1. Idiogram giving the morphological details of the pachytene chromosomes of *Solanum integrifolium*.

The evolutionary significance of these features and the cytological affinities of S. *integrifolium* to other species will be considered elsewhere with similar data for other taxa of the group and the F_1 hybrids involving this species.

We are thankful to the C.S.I.R. for the award of Junior Research Fellowship to one of us (P.B.K.), and to Prof. P. V. Bhiravamurthy, Andhra University, for providing us with the seed material.

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---

1. Magoon, M. L., Ramanujam, S. and Cooper, D. C., *Caryologia*, 1962, **15**, 151.
2. Rao, B. G. S., *Eco. Bot.*, 1972, **26**, 189.
3. —, *Ibid.*, 1975, **29**, 79.

ACCUMULATION OF STARCH IN RICE LEAVES INFECTED WITH *HELMINTHOSPORIUM ORYZAE*

ACCUMULATION of starch around the infection court has been reported to be a common phenomenon in obligate parasites[1–3], but as far as facultative parasites are concerned no precise knowledge is available in this context. In brown spot disease of rice incited by *Helminthosporium oryzae*, accumulation of starch around the diseased areas was observed.

TABLE I

Variation of starch accumulation in rice leaves infected with Helminthosporium oryzae

		Concentration of accumulated starch following infection		Diurnal variation of starch accumulation index 120 hours after inoculation		
Leaf position	Variety	72 h	120 h	6 A.M.	10 A.M.	6 P.M.
Topmost leaf	Benibhog	+	+	1·2	1·85	1·6
	CH 13	+	++	1·65	2·5	2·3
Second leaf from top	Benibhog	+	++	1·5	2·4	2·05
	CH 13	++	+++	2·0	3·2	2·8

+ = Concentration.

Two weeks old seedlings of two varieties of rice namely resistant CH13 and susceptible Benibhog were inoculated with the conidial suspension of *H.oryzae* grown on potato dextrose agar at 27 ± 1°C for 10–15 days. Freshly infected leaves were boiled in 80% ethanol for 20 minutes to remove chlorophyll· Achlorophyllous leaves were immersed in iodine-potassium-iodide reagent (1·5 g potassium iodide and 0·3 g crystal iodine in 100 ml distilled water) for 30 minutes. Presence of starch was detected by blue colour reaction. Concentration of starch was measured colorimetrically and 'starch accumulation index', *i.e.*, the ratio of area of starch accumulation to the spotted area was measured with the help of stage and ocular micrometer.

Starch accumulation increased with expansion of lesion and its maturation. Maximum concentration of starch accumulation was observed 120 hours after inoculation. Diurnal variation in 'starch accumulation index' was measured at 120 hours after inoculation. Data presented in Table I showed that starch accumulation index was greater at 10 A.M. in comparison with the value at morning and evening. Concentration of accumulated starch and starch accumulation index were greater in the resistant host in comparison to susceptible host. But starch accumulation was found to be greater in the second leaf than in the topmost leaf of both the varieties, though the topmost leaf is considered to be comparatively resistant,

Several factors like increased translocation, alteration in metabolic activity or decrease of β-amylase activity might individually or collectively play an important role. In their studies with enzymatic changes in *Helminthosporium* induced brown spot disease, Shishiyama *et al.*[4] observed increased catalase and decreased β-amylase activity in rice leaves following infection, which might influence accumulation of starch.

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West Bengal, *December* 3, 1976.

---

1. Allen, P. J., *Am. J. Bot.*, 1942, **29**, 425.
2. Garwitz, D. L. and Durbin, R. D., *Phytopathology*, 1960, **50**, 636.
3. Inman, R. E., *Ibid.*, 1962, **52**, 1207.
4. Shishiyama, J., Oguchi, T. and Akai, S., *Ann. Phytopath. Soc. Japan*, 1968, **34**, 16.

TOBACCO WILDFIRE DISEASE IN INDIA

DURING the 1976 growing season, a leaf disease of tobacco (*Nicotiana tabacum* L.) has been the cause of much concern to tobacco growers in Coimbatore District because of its destructiveness. Diseased specimens were brought to this department by tobacco cultivators. The apparently water-soaked margin of the diseased areas, the tissues of which, upon microscopic examination, were found to be teeming with bacteria, suggested that the disease was probably of bacterial origin. It is the present purpose of this report to adequately describe the disease and its causal organism.

The disease was observed to cause large necrotic lesions in the leaf with water-soaked areas along the midrib and veins. The infected leaves were distorted with dead rotten areas presenting a ragged appearance (Fig. 1–B). Such diseased material was macerated, diluted with sterile distilled water and used as inoculum in the preliminary inoculations. The causal organism was isolated under asceptic conditions in nutrient agar and purified subsequently by dilution plate method. Artificial inoculation with the organism produced water-soaked areas along the midrib and veins (Fig. 1–A) as observed under field infections (if heavy cell concentration was used in inoculum) and also lesions consisting of a small central dead area surrounded by a broad yellow halo[3]. Under artificial conditions, the lesions required about a week to develop fully. In repeated pathogenicity tests the organism produced similar symptoms on tobacco. Inoculation of the pathogen upon *Capsicum annuum*, *Arachis hypogaea*, *Solanum melongena* and *Lycopersicum esculentum* gave negative results.

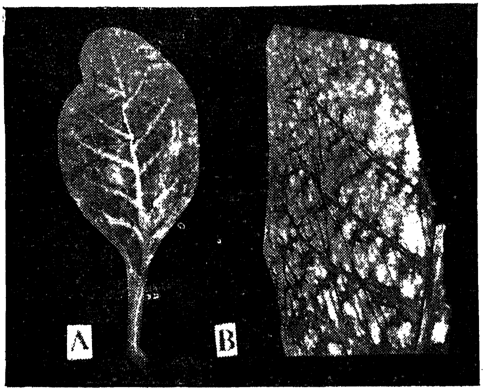

FIG. 1 A–B

The pathogen is a rod-shaped bacterium with size of the cells varying from $2 \cdot 0$ to $4 \cdot 5 \times 0 \cdot 9$ to $1 \cdot 6\,\mu$ and average size being $3 \cdot 2 \times 1 \cdot 1\,\mu$. The organism is motile by a single polar flagellum. It is gram negative and, however, is not acid fast. It produces acid but not gas in sugars like glucose, galactose, fructose, xylose, sucrose, glycerol and mannitol; does not produce indole; produces a diffusable green, fluorescent pigment in King's medium B[6]. It is oxidase negative but liquefies gelatin; the organism does not hydrolyse starch but reduces nitrates to nitrites. The process of denitrification is rather difficult to demonstrate in yeast extract-peptone broth but can be readily detected in a synthetic medium[4]. From the above description of this phytopathogenic bacterium and from its capability to initiate disease symptoms on tobacco alone, the organism is identified as *Pseudomonas tabaci* (Wolf and Foster) Stevens[1,2,5], the incitant of tobacco wildfire disease. The authentic culture has been deposited at Type culture collection at Indian Agricultural Research Institute, NewDelhi and in the type culture collection at the Tamil Nadu Agricultural University (item No. 56). This appears to be the first time this pathogen has been found in India.

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1. Wolf, F. A. and Foster, A. C., *Science*, 1917, **46**, 361.
2. — and —, *Jour. Agr. Research*, 1918, **12**, 449.
3. Clayton, E. E., *Ibid.*, 1936, **52**, 239.
4. Burkholder, W. M. and Starr, M. P., *Phytopathology*, 1948, **38**, 498.
5. Elliot, C., *Manual of Bacterial Plant Pathogens*, Chronica Botanica Co., Waltham, Mass., U.S.A., 1951.
6. King, E. O., Ward, M. K. and Raney, D. E., *J. Lab. Clin. Med.*, 1954, **44**, 301.

A COMPARATIVE STUDY OF EXTRACELLULAR ENZYMES PRODUCED BY PATHOGENIC FUNGI

SINCE not much is known about the enzyme make up of pathogenic fungi, particularly, the enzymes produced extracellularly, the present investigation was undertaken to study the different kinds of enzymes produced by some of these fungi in relation to their possible role in pathogenicity with the respective host. Extracellular amylase production has been reported from phytopathogenic fungi[1,2]. Cellulase enzymes necessary for the breakdown of cellulose of higher plant cell walls were also known to have been produced extracellularly by a number of phytopathogenic fungi[3-5]. Ribonuclease and deoxyribonuclease, though not related directly to the penetration of the pathogen into the host play an important secondary role of disrupting the protein metabolism of the host and host DNA degradation[6-8]. Little work however has been done so far, to correlate extracellular enzyme production by non-obligate fungi with pathogenicity.

The fungal strains (Table I) obtained from the Indian Agricultural Research Institute, New Delhi, India, were maintained on Czapek–Dox[9] medium and tested for their pathogenic activity prior to enzyme assay. Chemicals used were Na-salt of carboxymethyl cellulose (I.C.I. Ltd.), DNA of calf thymus and Yeast RNA (Nutritional Biochemicals Corporation), Soluble starch (E. Merck), Uranyl acetate (B.D.H.).

The cultures were grown in 100 ml capacity Erlenmeyer flasks containing 25 ml of Peptone-Glucose medium[10] for 10 days at 22° C and 30° C. The supernatant clear culture filtrates obtained after the removal of mycelial debris by

TABLE I

Enzyme activity (zone in mm ± S.E.) of different pathogenic fungi

Organisms	Enzyme activity*							
	Amylase		Cellulase		DNase		RNase	
	22° C	30° C	22° C	30° C	22° C	30° C	22° C	30° C
Alternaria brassicola	–	21·00±1·20	15·50±0·60	13·25±0·39	+	–	14·25±0·39	13·75±0·45
Alternaria crassa	–	–	12·00±0·12	21·00±0·99	–	–	–	–
Alternaria solani	17·90±0·25	13·66±0·53	–	–	–	–	–	14·50±0·81
Aspergillus fumigatus	16·37±0·35	23·25±0·21	16·50±0·30	15·50±0·91	23·25±0·80	–	26·50±0·90	16·75±0·43
Curvularia lunata	18·00±0·12	19·00±0·81	20·00±0·75	31·25±0·70	–	–	–	–
Fusarium oxysporum	–	–	–	14·50±0·72	–	–	–	–
Fusarium udum	–	–	–	–	–	–	17·77±0·20	19·25±0·25
Helmintho-sporium sativum	15·37±0·43	17·50±0·32	20·25±1·02	14·00±0·05	13·25±0·23	–	15·75±0·62	13·75±0·53
Helmintho-sporium oryzae	16·87±0·32	18·65±0·30	21·40±1·02	–	15·13±0·54	–	16·00±0·50	13·63±0·23
Rhizoctonia bataticola	–	–	–	18·50±0·09	–	–	18·80±0·53	15·50±0·32
Rhizoctonia solani	14·50±0·08	–	16·50±1·12	17·50±0·07	–	–	16·75±1·02	14·50±0·83
Ustilaginoidea virens	20·25±1·12	22·60±0·04	22·25±0·23	16·00±0·33	–	–	25·25±0·32	15·00±0·12

* Zone of clearing in mm; + presence of activity; – absence of activity.

centrifugation were used to assay for amylase, cellulase, deoxyribonuclease, and ribonuclease enzyme by cup-plate assay method[11]. Ten replicates were taken for each enzyme of a series. The uninoculated broth tested similarly served as control. Enzymes were assayed using 0·1 ml quantity of the supernatant culture broth pipetted to 7 mm diameter cup in each test plate containing 20 ml of the medium.

For the assay of amylase nutrient, agar with 1% starch was used[12]. The plates were incubated for 2 h at 37° C and then developed with Gram's Iodine solution[12]. Cellulase was assayed using carboxymethyl cellulose in acetate buffer of pH 5·6[12]. Plates were incubated for 18 h at 37° C and then developed with chloroiodide of zinc solution[13]. For RNase and DNase assay, the medium was plated after adding yeast RNA and calf thymus DNA respectively, incubated for 2 h at 37° C and then developed with uranyl reagent[10]. In each case the diameter of the zones of clearing were measured in mm.

Of the 12 pathogenic strains studied, 8 gave positive results for amylase, 10 for cellulase, 3 for DNase and 9 for RNase (Table I).

It was observed that the effect of temperature on enzyme production and the quantity produced varied from organism to organism. In some cases a lower temperature of 22° C was proved conducive to enzyme production while in others a higher temperature of 30° C was found more effective.

In *Alternaria* sp. at 28–30° C encouraged amylase and cellulase production. In the case of *Fusarium* sp. 17–29° C was reported to be favourable for disease development[14]. Optimum production of cellulase and ribonuclease was obtained at a temperature of 22° C in these species. Also, it has been found that seedlings emerge slowly between 16–24° C and the maximum seedling infection by

Helminthosporium sp. is induced between 16–24° C[15]. In the present study it was found that optimum cellulase, DNase and RNase production occurred at 22° C (Table I) which falls within the temperature range optimum for disease development. These studies reveal that the significant activities of various enzyme systems indicate encouraging possibilities for their quantitative estimation in relation to pathogenecity.

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1. Das Gupta, S. N. and Verma, J. P., *Curr. Sci.*, 1961, 30, 450.
2. Ksendova, E. N., *Byulleten Vsesoyuznogo Nauchno Isseldovatel'skogo Instituta Zashchity Rastenii*, 1974, 27, 72.
3. Higa, H. A., O'Neill, R. D. and Jennison, M. W., *J. Bacteriol.*, 1956, 71, 382.
4. Husain, A. and Rich, S., *Phytopathology*, 1958, 48, 316.
5. Arirze, A. E., Naqvi, S. H. and Ekundayo, J. A., *International Biodegradation Bull.*, 1976, 12 (1), 15.
6. Verma, J. P., *Mycopathol. et Mycol. Appl.*, 1963, 20 (3–4), 337.
7. Glitz, D. C. and Dekker, C. A., *Biochemistry*, 1964, 3, 1391.
8. Heitefuss, R., *Ann. Rev. Phytopathol.*, 1966, **4, 221.**
9. Basu, S. N., Bose, R. G. and Bhattacharyya, J. P., *J. Sci. Ind. Res.* (*India*), 1955, 14 **C**, 46.
10. Berkenkamp, B., *Can. J. Microbiol.*, 1973, 19, 1431.
11. Dingle, J., Reid, W. W. and Solomons, G. L., *J. Sci. Fd. Agric.*, 1953, 4, 149.
12. Seeley, H. W. and Vandemark, J. P., *A Laboratory Manual of Microbiology*, W. H. Freeman and Co., 1962, p. 195.
13. Sass, E. J., *Botanical Microtechniques*, Oxford and IBH Publishing Co., 1967, p. 97.
14. Mundkur, B. B., *Ind. J. Agric. Sci.*, 1935, 5, 609.
15. Singh, R. S., *Plant Diseases*, Oxford and IBH Publishing Co., 1971, p. 330.

TOXICITY OF A NEW RODENTICIDE, VACOR TO LESSER BANDICOOT RATS, *BANDICOTA BENGALENSIS*

DAMAGE, losses and disease attributable to rodents in India are well documented[1,2]. *B. bengalensis* is one of the most common agricultural and commercial pests, ranging over almost the whole of India except for the drier regions of Rajasthan and Gujarat[3]. Despite recognition of the serious losses to this species, little information is available on its susceptibility to rodenticides.

Of acute poisons used against *B. bengalensis*, only zinc phosphide and aluminium phosphide are effective and readily available; and of these, only zinc phosphide can be given with bait[3]. Various chronic anticoagulants have also been tested[4,5], but *B. bengalensis* shows considerable variation in susceptibility to warfarin and difenacoum[6,7]. If only anticoagulants are used to control *B. bengalensis*, problems of resistance may become important[6,8].

There is thus urgent need for the development of new effective acute poisons as an alternative to zinc phosphide. Vacor (N-3-pyridymethyl N^1-*p*-nitrophenyl urea) is such a rodenticide recently developed by Rohm and Haas, USA; it is thought to be a nicotinamide antagonist, and is highly effective against a wide variety of rodents[9]. Laboratory tests were therefore made of its effectiveness against *B. bengalensis*; field trials in rice and sugarcane are also being conducted.

B. bengalensis were caged singly in metal cages, 60 × 30 × 30 cm, with water always available. For four days, rats were fed weighed amounts of rice flour + groundnut oil (95 : 5 by weight); on the fifth day, Vacor was added to the food in concentrations of 1% or 0·25% for different groups. Consumption corrected for spillage was measured each day to 0·1 g and converted to 100 g body weight for analysis.

Results on the toxicity of Vacor are summarised in Table I. All rats died in less than 18 hours; symptoms were first noticed after 2–3 hours and involved a noticeable reduction in general activity, with later partial paralysis of the hind legs, pulmonary distress and coughing. Dead rats were often found with fore and hind limbs held rigid out from the body so that the corpse was supported off the cage floor.

In a second experiment, three male and two female *B. bengalensis* (mean weight 170 g) were caged singly as before and fed rice flour + 5% groundnut oil. After consumption had stabilized, rats were weighed, starved for 30 hours, and then

TABLE I

Toxicity of Vacor to lesser bandicoot rats

(means ± S.E.)

Concentration of Vacor %	Rats M	Rats F	Weight, g (Range)	Mortality %	Food eaten g/100 g body wt. (Range) Unpoisoned	Food eaten g/100 g body wt. (Range) Poisoned	Poison eaten mg/kg body wt. (range)
0·25	2	3	185 (159–215)	100	6·0±0·4 (5·1–7·4)	1·3±0·3 (0·9–2·5)	33·3±7·2 (23·3–61·7)
0·25	3	2	341 (213–392)	100	5·3±0·3 (4·6–6·3)	1·4±0·2 (0·7–1·8)	34·8±5·3 (17·5–46·1)
1·0	2	3	293 (224–396)	100	6·0±0·3 (5·4–7·0)	1·3±0·2 (0·6–2·0)	125·8±23·0 (57·4–200·7)

fed 2 g of the food, mixed with Vacor at a concentration of 20 mg/kg initial body weight. Thirty minutes after the poisoned food was put in the cages, all rats had eaten all of the food. All five rats died overnight; conditions of death were as noted above.

The LD_{50} for Vacor is obviously less than 20 mg/kg body weight, and rats fed 1% Vacor ate, on average, more than six times that amount of poison. Vacor is also about twice as toxic as zinc phosphide; and when mixed with food at 1% concentration, it has about double the intake of zinc phosphide bait of the same concentration (unpublished work). No problems of acceptance of Vacor baits were observed during any tests, although such problems have been noted for other species[9]. The present findings suggest that 1% concentration of Vacor should be effective against lesser bandicoot rats; a recent field trial in urban Calcutta, using 2% Vacor against a mixed population of *R. rattus* and *B. bengalensis*, demonstrated as effective control as that using zinc phosphide[10]. Before a general recommendation for field use can be made, however, information is needed on the toxicity of Vacor to the other principal Indian rodent pest species[3]. This new effective acute rodenticide should also prevent the development of poison and bait-shy residual populations during control campaigns when used as an alternative in conjunction with zinc phosphide[2].

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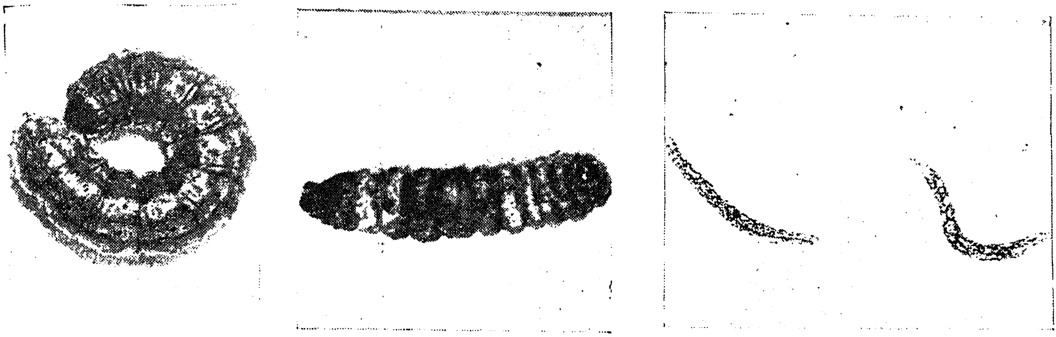
1. Patnaik, K. C., *Indian Rodent Symposium*, Calcutta, 1966, p. 4.
2. Prakash, I., *Central Arid Zone Research Institute*, Monograph No. 4, Rodent Pest Management.
3. Barnett, S. A. and Prakash, I., *Rodents of Economic Importance in India*, Arnold-Heinemann, New Delhi, 1975.
4. Deoras, P. J., *Curr. Sci.*, 1965, 34, 348.
5. —, *Ibid.*, 1967, 36, 207.
6. Greaves, J. H. and Rehman, A. B., *Journal of Hygiene*, 1977, 78, 75.
7. Renapurkar, D. M., Menon, T. U. K., Bhat, U. M. and Sant, M. V., *Pesticides*, 1973, 7, 23.
8. Fernando, H. E., Hawamoto, N. and Perera, N., *Plant Protection Bulletin*, F.A.O., 1967, 15, 32.
9. Marsh, R. E. and Howard, W. E., *W.H.O. Vector Biology and Control*, 1975, 75, 563.
10. Ramakrishnan, L. and Suchak, P. B., *All India Rodent Symposium*, Sidhpur, 1975.

ISOLATION OF AN ENTOMOPHILIC NEMATODE FROM POTATO CUTWORMS

DURING 1973–74 a survey for natural enemies of potato cutworms was conducted in Simla (H.P.) and Jullundur (Punjab). In April 1974, a total of 4,160 caterpillars of *Agrotis ipsilon* Hufn. was collected from Jullundur, of which 320 were infected with an entomophilic nematode (*Neoaplec-*

tana sp.). Some of the *A. segetum* caterpillars were also found infected with this nematode. Cutworms dying of nematode infection exhibited similar symptoms as caused by bacterial septicemia. As most of the caterpillars were in their final instars, mortality (due to this nematode) in their earlier instars could not be determined.



FIGS. 1–3. Fig. 1. Healthy caterpillar of *Agrotis segetum*. Fig. 2. Caterpillar of *A. segetum* infected with lower concentration of *Neoaplectana* sp. Fig. 3. *Neoaplectana* larvae.

For obtaining nematode culture, infected cutworms were washed with sterilized water and kept in a water trap. The nematode larvae inside the host migrated through water to the bottom of the basal dish of the trap. These larvae were collected daily.

For pathogenicity test, eight earthen pots were taken, of which four pots were filled with sterilized sand and four with garden soil. Twenty larvae of *A. segetum* in their third instar were released in each pot. The cutworms were fed on potato leaves. Four pots (2 with sand and 2 with garden soil) containing potato leaves and cutworms were sprayed with nematode suspension and the remaining set was sprayed with water to serve as control. The pots were covered with perforated polyethylene sheets. Food was changed daily. Cutworms in the pots were not disturbed and at the end of the experiment total number of moths emerged in each pot was noted. From the treated pots no moths emerged, but from control all the caterpillars gave rise to moths.

Some of the cutworms infected with this nematode were kept individually in petri dishes for further observations. From the heavily infected cutworms, the larvae (Fig. 3) after eating away the internal contents of the host started emerging and spreading all around the body of the host. Within a period of about 10 days these nematodes consumed all the body contents of the host leaving behind the chitinous head capsule and cuticle. The cutworms which ingested less number of nematodes showed visible symptoms of infection, *viz.*, white colonies of nematode on the underside of the abdominal segments (Fig. 2), and such caterpillars took unusually longer time to die.

The pathogenicity of this nematode was also tested on the field collected caterpillars of *A. ipsilon* Hufn., *Amathes c-nigrum* L. and white grubs. All the caterpillars died, but in case of white grubs only 75% mortality was caused by this nematode.

Isolation of this entomophilic nematode (*Neoaplectana* sp.) from potato cutworms (*A. ipsilon* and *A. segetum*) is a new record from India. However, success has earlier been reported for the control of various pests of other crops by means of Neoaplectanid nematodes[1-6].

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1. Duttky, S. R. and Hough, W. S., *Proc. ent. Soc. Wash.*, 1955, 57, 244
2. Israel, P., Rao, V. R. V. J.; Prakash Rao, P. S. and Varma, A., *Curr. Sci.*, 1969. 38, 390.
3. Rao, V. P. and Manjunath, T. M., *Indian Fungi*, 1966, 16, 43.
4. Rao, Y. R. V. J., Prakash Rao, P. S., Varma, A. and Israel, P., *Indian J. Ent.*, 1971, 33, 215.
5. Schmiege, D. C., *J. Econ. Ent.*, 1963, 56, 427.
6. Singh, J. and Burdhan, A. K., *Curr. Sci.*, 1974, 43, 622.

CONCERNING THE INFLUENCE OF DEEP WATER AND FIELD CONDITIONS ON THE DEVELOPMENTAL PROCESSES OF THE FLOATING RICE (*ORYZA SATIVA* L.)

THE developmental process of floating rice varieties comprises of a number of important growth phases. Two separate experiments were simultaneously conducted one under usual field condition, and the other under deep water condition to study this aspect at the Rice Research Station, Chinsurah, West Bengal. The normal cultivation methods were practised under field condition, *i.e.*, maintaining water level at 6–8 cm under different growth phases. In the second case the plants were grown in suitably constructed tank in a low lying area, and connected with the main drainage channel so that after 2–3 showers of the monsoon, the tank had sufficient water. Data on the variations of water level at various growth phases in the tank and the field are given below:

Deep Water condition		Field condition	
Growth phase	Water level (cm)	Growth phase	Water level (cm)
Germination	Wet	Germination	Wet
Seedling	5–22	Seedling	Wet
Basal tillering	20–50	Basal tillering	6–8
Early culm elongation	42–182	Pre-emerging reproductive	6–8
Nodal tillering	125–190	Flowering	6–8
Late culm elongation	160–210	Ripening	1–8
Pre-emerging reproductive	200–210		
Flowering	132–190		
Ripening	124–142		

'Wet' 37 – 41% soil moisture

The newly developed photosensitive floating variety —'Jaladhi 2'—was grown both under deep water and the field conditions. The seeds were directly sown in wet soil towards the end of April. A diagrammatic representation of the relative lengths of various growth phases throughout the life period (240 days) of the floating rice variety under the field and the deep water conditions is provided in Fig. 1.

After 34 or 35 days from the date of sowing the basal tillers started growing in both treatments (Fig. 1). In general, when the plants attained an age of 45–50 days and were submerged with 40–45 cm, these started forming long internodes. Rapid axial growth of culm is associated with the vegetative phases under deep water condiition. In floating rice plants, the formation of extended internodes is independent of the panicle development and in the later stage only the 3 or 4 uppermost internodes elongated with unusual rapidity. However, under field conditions, the culm elongation and the panicle initiation occurred almost concurrently and the plants behaved similar to normal tall *indica* rice varieties (Fig. 1). It is a matter of common occurrence with many monocotyledonous plants that rapid extension of culm is closely associated with reproductive development[1–3]. The plants under deep water condition entered the nodal tillering phase following the early vegetative culm elongation phase immediately after the aerial (nodal) branching started. Nodal tillering appeared about 3 months after sowing and continued for about 4–6 weeks thereafter. The duration of this period is a varietal character. Under deep water condition all the various growth phases and subphases, *viz.*, basal tillering, vegetative culm elongation, nodal tillering and reproductive ones, are distinct and a knowledge of the various phases would be very useful. With reference to the developmental processes of floating rice plants under deep water condition (Fig. 1), the following phases and subphases could be distinguished: (i) the conpact crown or hard culm phase (*i.e.*, covering seedling and basal tillering phases) characterised by having unelongated internodes, a few basal tillers and of relatively long duration; (ii) the early vegetative culm elongation phase associated

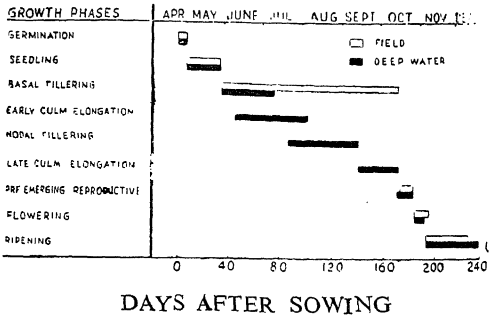

FIG. 1. Relative lengths of various growth phases of floating rice under the field and the deep water conditions.

with high extension growth of internodes with the axial development, very variable internodal length depending on the depth and duration of submergence and of long duration; (iii) the nodal tillering stage having emergence of aerial branches (nodal tillers) with relatively unaltered culm length and of very variable in number of nodal tillers depending upon the variable depths of submergence and of long duration; (iv) the late culm elongation phase characterised by a gradual increase in length of the internodes situated above the nodal tillers, their numbers dependent on the depth of submergence and duration; (v) the pre-emerging reproductive period associated with the sudden extension of 3–4 elongated uppermost internodes with the aerial development of panicles and (vi) the post emergence prolonged ripening phase. Two important points emerge from the data on growth processes: (i) variability of the different phases and subphases of the floating rice is relatively less marked under field condition and (ii) under deep water condition the vegetative culm elongation periods (early and late) is distinct with larger number of elongated internodes and nodal tillering. The above findings of developmental physiology may offer some scope to the rice workers for the improvement of floating rice varieties. The phasic development of the floating rice under deep water condition is different from ordinary rice culture.

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1. Aitken, Y., *Aust. J. agric. Res.*, 1961, **12**, 371.
2. Cooper, J. P., *J. agric. Sci.*, 1956, **47**, 262.
3. Kaufman, P. B., *Indian J. agric. Sci.*, 1960, **30**, 233.

THREE NEW RECORDS OF FOLIICOLOUS LICHENS FROM INDIA

RECENTLY Awasthi and Singh[1,2] (1972, 1973) and Singh[3] (1973) reported several foliicolous lichen taxa either as new species or new records from South Indian hills and Andaman Islands. However, very little is known about the foliicolous lichens from North-East India. To get more precise information from these parts, the author visited the state of Manipur during the months of October–November 1976, and collected more than 400 packets of different lichens from there. Critical studies on some foliicolous specimens resulted in three new records of lichens for India, which are described below.

ASTEROTHYRIACEAE

Calenia conspersa (Stirt.) Sant.

Thalli dispersed in small to big patches, 5–30 mm across, smooth, greenish-grey to whitish-grey; sometimes provided with white hairs; apothecia numerous, primarily immersed and closed but at maturity become prominent with a rounded open mouth, 0·1–0·25 mm in diam; disc invisible in young apothecia, but mature apothecia have yellowish-brown, deeply concave epruinose disc; exciple colourless, 5–7 μm thick below and laterally; hymenium colourless, 40–50 μm high; asci 8-spored; spores colourless, transversely 1-septate, ellipsoid, 7–9·3 × 2·5–3·5 μm; paraphyses branched and anastomosing.

The species known from America and Philippines is characterized by small circular apothecia with disc usually covered by the thalline margin, transversely 1-septate and size of spores. The spore size in our specimen is slightly smaller than the size of spores (8–12 × 2·5–4 μm) recorded for American and Philippines specimens (Santesson[4] 1952). It is a new generic record for India.

Manipur–Karang: Kabrulakha, *Singh* 54457 (CAL).

Gyalectidium rotuliforme Müll. Arg.

Thalli scattered in small circular to irregular patches, 1–3 mm in diam, usually containing 1–2 apothecia in the centre, smooth, greyish-white, with a single layered plectenchymatous cortex; apothecia immersed in the thallus but prominent, easily shed off from the thalli; yellowish-brown, 0·15–0·25 mm in diam; exciple colourless; hymenium colourless, 85–100 μm high with epithecial algal cells above; asci 1-spored; spores colourless, multicelled, muriform, oblong; 32–60 × 15–18 μm; paraphyses nonarticulate and branched.

The species distributed in the pantropical regions is characterized by the smooth thallus and presence of epithecial algae. The spore size in our specimen is smaller than the size of spores [50–80 × 14–25 (30) μm] reported by Santesson[4] (1952).

Manipur–Karang: Kabrulakha area, *Singh* 54458 (CAL).

Tricharia triseptate Sant.

Thallus occurs in small patches, smooth, greenish-grey to whitish-grey, provided with long tapering black hairs; apothecia few, present on the midrib and petiolar region of the leaf, light brown, transparent, sessile, constricted below, 0·2–0·3 mm in diam ; exciple colourless, prosoplectenchymatous ; hymenium colourless, 45–65 μm high ; asci usually immature, 8-spored ; spores small, not well developed, colourless, ellipsoid, transversely 3-septate, 9·12 × 3·5 μm ; paraphyses slightly branched.

The species so far known from America and Africa are characterized by the transversely 3-septate, spores.

Manipur-Oukhrul : 4-miles below Oukhrul, on way to Lamblangate, associated with Byssoloma species, *Singh* 54459 (CAL).

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Botanical Survey of India, K. P. SINGH.
Howrah, *May* 23, 1977.

---

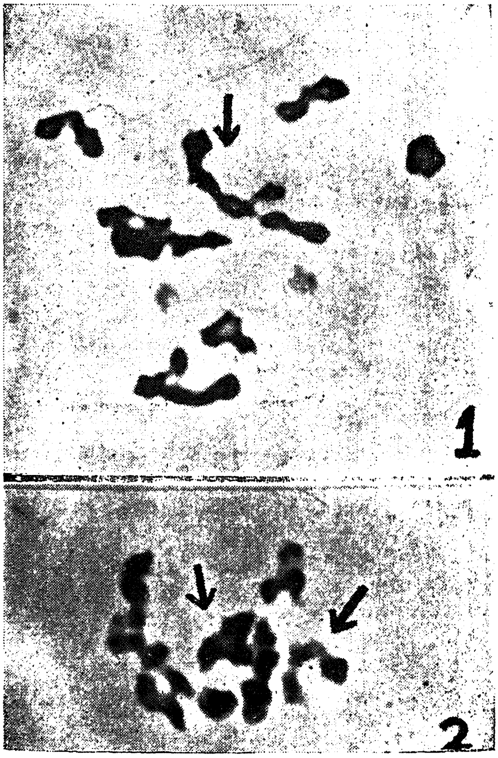
1. Awasthi, D. D. and Singh, K. P., *Norwegian J. Bot.*, 1972, 19 (3–4), 239.
2. — and —, *Geophytology*, 1973, 3 (1), 13.
3. Singh, A., *Rev. Bryol. et lichenol,* 1973, 39 (3), 479.
4. Santesson, R., *Symb. Bot. Upsal.*, 1952, 12 (1), 590.

CHROMOSOMAL REPATTERNING IN THE DIFFERENTIATION OF TWO SPINOUS SOLANUMS

PREVIOUS attempts at interspecific hybridization of some spinous solanums have yielded variable results, and tht conclusions arrived at on their cytogenetic affinities are uncertain[1,2]. To reevaluate the species relationships, we crossed seven species in different combinations following a modified technique[3], and obtained variable numbers of hybrids. The pairing behaviour of chromosomes at meiosis in these hybrids are being studied to assess the intergenomic homologies and the extent of chromosome repatterning in the divergence of the parental genomes. Our results on *Solanum surattense* Burm. *f* × *S. trilobatum* L. and its reciprocal are reported in this note. While the *S. trilobatum* parent was the same in both the crosses, two different plants of *S. surattense* (SS–1, SS–2) were used.

In the *S. surattense* (SS–1) × *S. trilobatum* (ST) combination 86 crosses were made and 37 fruits (43%) with an average of 112 seeds per fruit were realised. 83% of the seeds were healthy and 25 hybrids were raised. In the reciprocal cross (ST × SS–2) 109 crosses were made and 51 fruits (46·1%) were obtained, of which 43 had fleshy placenta with no seed ; the other 8 fruits had an average of 5 seeds from which 3 hybrids were obtained.

Meiotic studies in these F–1 hybrids revealed that the number of chromosomes involved in pairing was variable in the different PMCs analysed, and ranged from twelve bivalents per nucleus to only six bivalents accompanied by one or two rings or chains of three, four or five, with the rest remaining as univalents (Figs. 1 and 2). The univalent frequency ranged from twelve to as low as one per nucleus. The relevant data are summarised in Table I.



FIGS. 1–2. Fig. 1. PMC at diakinesis in the hybrid SS–1, × ST showing chain of four chromosomes (arrow) and ten bivalents (× 2,000). Fig. 2. PMC at diakinesis—metaphase-I showing two trivalents and eight bivalents (× 2,000).

The occurrence of 12 bivalents in 22 of 107 PMCs analysed in the SS–1 × ST hybrid clearly shows that each of the 12 chromosomes in the haploid set of the two genomes have sufficient homologies to pair and form chiasmata. This is also supported by the occurrence of 35 nuclei with $11_{II} + 2_{I}$, and 31 nuclei with 10 + univalents or higher associations. In other words, out of 107 PMCs, 10 or more bivalents are observed in 88 nuclei. At the same time rings or chains of

TABLE I

The different chromosome associations and their frequencies at Diakinesis and Metaphase–I in the F–1 of S. surattense × S. trilobatum *(SS–1 × ST) and its reciprocal (ST × SS–2)*

Material SS–1 × ST		Chromosome associations					Material ST × SS–2	
Frequency at				Rings or chains of			Frequency at	
Diakinesis	Metaphase–I	Univalents	Bivalents	III	IV	V	Diakinesis	Metaphase–I
1		5	7			1		
1		2	6	2	1			
			8		2			1
		5	6	1	1		1	
	1		9	2				
		4	7	2				1
		6	6	2			1	
6	3		10		1		1	2
1	1	2	9		1			1
3		4	8		1			2
8	3	1	10	1				
4	2	3	9	1				3
2		5	8	1				1
13	9		12				1	1
29	6	2	11				4	11
11		4	10				5	6
3		6	9				3	7
		8	8				2	5
		10	7				3	3
		12	6					1
82	25	TOTAL					21	45

three, four or five chromosomes are met with in 36 PMCs. These higher chromosome associations suggest that a few chromosomes in one of the genomes have homeologies to more than one chromosome in the other. Apparently the two species share a number of common loci that permit their chromosomes to pair and form chiasmata. The quantitative data on the F–1 of the reciprocal (*viz.*, ST × SS–2) are somewhat different from those of SS–1 × ST. But qualitatively they are nearly the same, and the conclusions to be drawn on their intergenomic homeologies remain unaltered.

In an earlier report[4] formation of only bivalents with variable numbers of univalents was recorded in a sample of 26 PMCs analysed for the F–1 of *S. surattense* × *S. trilobatum*. Possibly sampling of a larger number of nuclei would have revealed the higher chromosome associations observed by us. This assumption is justifiable from the fact that in our own analysis of 107 nuclei, bivalents with variable numbers of univalents were seen in 71 of them.

Cytological data given above provide conclusive evidences for the two species *S. surattense* and *S. trilobatum* sharing certain common loci but with considerable chromosome repatterning in their divergence. Since the cross both-ways and produce viable F–1 s, though only on artificial pollinations, the isolation mechanisms in them are not as fully developed as the absence of natural hybridization between them would suggest.

One of us (SVR) is thankful to the CSIR for the financial assistance.

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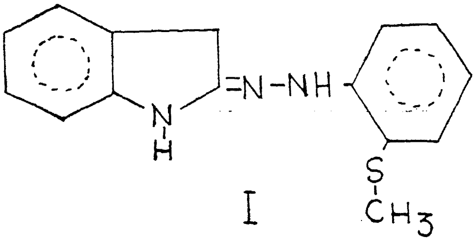
---

1. Magoon, M. L., Ramanujam, S. and Cooper, D. C., *Caryologia*, 1962, 15, 151.
2. Rao, B. G. S., *Eco. Bot.*, 1972, 26, 189.
3. Veerabhadra Rao, S. and Rao, B. G. S., *Curr. Sci.*, 1977 (In press).
4. Rajasekaran, S., *Annamalai Univ. Agric. Res. Annual*, 1969, 1, 4960.

A NOTE ON DIHYDROINDOLE PHENYL HYDRAZONE (DIPH)—A NEW HETEROAUXIN

RECENTLY Sarma and Barooah[1] have reported the formation of a new dihydroindole phenyl hydrazone (I) during the diazo coupling of indole with a diazonium chloride obtained from *o*-amino thio anisole. Since the early synthesis of indole acetic acid (IAA) involved the diazo coupling of benzene diazonium chloride with diethyl-A-acetyl glutarate[2] this new compound was tested for auxinal properties. It showed positive root initiation and development activity when applied to the terminal cuttings of tomato, dahlia, juniperus and thuja. In this note the auxinal activity of the new compound (DIPH) is reported.

Diazo coupling of indole with the diazonium salt obtained from *o*-amino thio anisole was carried out by the method of Avrameonko, Nagina and Suvorov[3]. The pH of the solution was restricted to 5. The resulting precipitate was chromatographed on a column of alumina using benzene as eluent. The structure of the main compound obtained, was elucidated by u.v., i.r. and p.m.r. spectral analysis and confirmed by elemental analysis. The structure corresponds to a dihydroindole phenyl hydrazone (Fig. 1).



I

FIG. 1

For determining auxinal activity, terminal cuttings of tomato, dahlia, juniperus and thuja were treated with DIPH by the soaking method[4] at 250 ppm. The results are recorded in Table I.

TABLE I
No. of Samples treated 10.

Plant Cutting	% rooting	Mean length in cm.	Auxin used
Tomato	90	7·2	IBA
Dahlia	70	5·7	do.
Thuja	20	5·2	do.
Juniperus	30	3·5	do.
Tomato	60	9·7	DIPH
Dahlia	50	10·2	do.
Thuja	20	4·5	do.
Juniperus	50	5·7	do.

N.B.—Untreated cuttings failed to produce roots.

It is clear from Table I that DIPH has auxinal activity on the development of roots in cuttings. Although IBA induced a larger number of roots than DIPH their length did not exceed those produced by treatment with DIPH.

It has been reported[5–10] that the auxinal activity of indole acids is due to an indole acid phenol complex which forms at the base of the cuttings. The phenol fragment being produced by the plant itself.

Experimental

Preparation of DIPH.—0·7 g of *o*-aminophenyl methyl sulphide hydrochloride was dissolved in 20·0 ml water and 0·5 ml conc. HCl. To it 0·35 g of a saturated solution of sodium nitrite was added at 0° C and allowed to stand for 15 mini This diazo solution was added drop by drop into a well stirred suspension of 0·59 g of indole in 50·0 ml. of water containing 0·05 g NaOH and 0·5 g of Na_2CO_3. The temperatire was maintained at 0° C and the addition was completed within one hour. Stirring was continued for one hour more until coupling was complete. The solid obtained was filtered, dried and column chromatographed over alumina using benzene as eluent (Yield 0·52 g; m.p. 192° C).

Application of DIPH.—The basal end of cuttings was treated with DIPH at 250 ppm for 24 hr. After treatment the cuttings were inserted into rooting medium immediately. Direct sunlight was avoided to prevent wilting.

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---

1. Sarma, P. K. and Barooah, S. K., communication sent to *J. Am. Chem. Soc.*
2. Ecuyer, L., *J. Chem. Soc.*, 1934, p. 1901.
3. *cf.* Avrameonko, V. G., Pershin, G. N., Nagina, V. D., Zykov, T. N. and Suvorov, N. N., *Pharm. Chem. J.*, 1970, 6, 317.
4. Hartmann, H. T. and Kester, D. E., *Plant Propagation—Principle and Practice*, Prentice-Hall of India (Pvt.) Ltd., 2nd edition, 1972. p. 306.
5. Cooper, W. C., *Bot. Gaz.*, 1938, **99**, 599.
6. Thimann, K. V. and Delisee, A. L., *Jour. Arnold Arb.*, 1936, **20**, 116.
7. —, and —, *Ibid.*, 1942, **23**, 103.
8. Bonillene, R. and Bonilenne, W. M., *Rept. 14th Int. Hort. Cong.*, 1955, **1**, 231.
9. Hess, C. E., *Rept. Proc. 16th Int. Hort. Cong.*, 1962, p. 382.
10. Fadl, M. S., *Plant Physiol.*, 1967, **42**, 541.

CHANGES IN PROTEIN CONTENTS OF URAD SEEDS (*PHASEOLUS MUNGO* L.) DUE TO FUNGAL FLORA

FUNGI play a vital role in determining the storage life of seeds. Urad (*Phaseolus mungo* L.) seeds are very rich in vegetable proteins. A number of fungi belonging to diverse taxonomic groups were isolated and identified from these seeds. The present investigation is mainly concerned with the changes in protein contents of the above-mentioned seeds during fungal infestation.

Seeds were randomly selected and divided into two lots. One was incubated in glass chamber with 100% humidity while the other was treated as control without any source of moisture. On the 7th, 14th and 21st days, 0·3 g of the seeds were finely crushed and powdered in homogenizers by adding 6 ml of 0·2 N NaOH. The protein was precipitated by 10 ml of 5% cold trichloroacetic acid and subsequently centrifuged and the residue was used for protein estimation. The supernatant was dried on a water bath and digested separately. From the residue, the percentage of total proteinaceous nitrogen was calculated by using Markham[2] still distillation apparatus. It was multiplied by the factor 6·25 to obtain the protein percentage. Percentage of soluble non-proteinaceous nitrogen was calculated from the supernatant stock by similar procedure. Protein estimation of the control stock was performed after 21 days of incubation. Results are summarised in Table I.

TABLE I

Showing changes in protein contents of urad seeds due to fungal infestation

	Days of incubation	% of protein nitrogen	% of protein (approx.)	% of soluble nitrogen
1.	0 day	3·41	21·34	1·5
2.	7 days	3·46	21·62	0·6
3.	14 days	2·61	16·31	2·0
4.	21 days	1·94	12·14	2·44
*5.	Control	3·25	20·31	1·55

* The protein contents of the control remained almost the same for the entire period as it was not exposed to moisture.

Table I shows that the protein contents either remained unchanged or were only slightly altered during first seven days which obviously reflects the mild fungal activity in beginning. There was a gradual depletion of protein in the seeds from the second phase which continued upto the end of the incubation. With the decrease of the proteinaceous nitrogen the soluble nitrogen increased which can be attributed to hydrolysis of the seed protein due to fungal activity. The control seeds did not express any pronounced variation in protein contents which is obviously due to non-availability of moisture for fungal activity. Earlier Christensen and Kaufmann[1] as well as Neergaard[3] mentioned that moisture enhances the fungal activity resulting in rapid seed deterioration.

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---

1. Christensen, C. M. and Kaufmann, H. H., *Grain Storage. The Role of Fungi in Quality Loss*, University of Minnesota Press, Minneapolis, 1969.
2. Markham, R., *Biochem. J.*, 1942, 36, 790.
3. Neergaard, P., *Seed Pathology*, Vol. I, The MacMillan Press Ltd., London, 1977, p. 285.

A CYTOLOGICAL ASSESSMENT OF SPECIES DELINEATION OF TWO CERAMIUMS (RHODOPHYCEAE)

Two *Ceramium* species occurring on Visakhapatnam coast[1], *viz*., *C. fimbriatum* Setch and Gard., and *C. gracillimum* (Kutz.) Griff. *et* Harv. var. *byssoideum* (Harvey) Mazoyer, are taxonomically distinguished by the presence of ephemeral and vesicular lateral hairs arising from cells of the cortical bands in *C. fimbriatum* and their absence in *C. garcillimum* var. *byssoideum*[1,2]. The two species are dioecious and are natural outbreeders, and occur as freely intermixed populations in some habitats.

As many simple exomorphic characters (like the presence or absence of lateral hairs) are known to be the alternate allelic expressions of one or more genes in many higher plant species, it was tempting to regard the intermixed populations of the two ceramiums as representing a single species, possibly segregating for the occurrence or not of the lateral hairs. An obvious way to verify such an assumption was to subject the material to genetic tests. Unfortunately, the two species are microscopic forms, and a meaningful analysis of the population structure or the segregating patterns through successive generations in their natural environs is not feasible. The only recourse, therefore, is to study the cytology of the two species and draw such conclusions as the data would permit. Since such information is not available in the literature[3,4] we studied some cytological aspects in these two species, and our results on *C. fimbriatum* have been reported earlier[5]. Data on the chromosome numbers and their behaviour at meiosis in *C. gracillimum* var. *byssoideum* are presented now, and on this basis, the validity of the taxonomic separation of these two 'forms' into different species (based on the presence or absence of lateral hairs) is considered.

Following the customary procedures of fixation and smearing[5], observations were made on dividing tetraspore mother cells (TMCs), and the diploid chromosome number and their behaviour at diakinesis were ascertained. The chromosome number varied from $2n = 64$ to 114. In a total of 32 TMCs analysed, the frequencies of nuclei with different chromosome numbers are :

2 *n* Chromosome No.	64	78	82	84	86	88	92	98	100	104	108	110	114
No. of Nuclei	1	2	1	**14**	1	2	1	2	2	2	1	2	1

Besides the chromosome numbers, the chromosome associations and their frequencies also varied in different nuclei. Higher associations, ($3_{\text{iv}} + 3_{\text{iii}}$ or more frequently $2_{\text{iv}} + 1$ or 2_{iii} besides bivalents and univalents per nucleus) were observed in 16 (50%) TMCs. Similarly univalents occurred in high frequencies, ranging from 12 to 27 per nucleus. Considering that 14 of the 32 cells analysed showed $2n = 84$, it is assumed that this represents the normal diploid chromosome number for the species, and that the others with lesser or higher numbers represent aneuploid derivatives of this or a common ancestral form.

As compared to *C. fimbriatum* with $2n = 42$, *C. gracillimum* var. *byssoideum* is cytologically distinct in its higher chromosome number, and higher frequencies of polyvalents and univalents at meiosis. The entire material without the lateral hairs showed chromosome numbers ranging from $2n = 64$ to 114 and none with 42 were encountered. If the presence or absence of the lateral hairs, the taxonomic criterion were due to allelic differences in the same species, and the population was segregating for this feature, one would expect forms *without lateral hairs* to have $2n = 42$ chromosomes (cf. *C. fimbriatum*) ; likewise, forms *with lateral hairs* should have the $2n$ chromosome number of 64 to 114 (majority with $2n = 84$) as in the present material. Such is not the case. It is, therefore, to be inferred that the taxonomic separation of the two species, on so simple a criterion, is justifiable, and that this feature is not due to allelic differences in a common genome, but concerns those of the two genomes in their entirety, and that the two species are distinct in their cytogenetic aspects. Further, while *C. fimbriatum* is a stabilized polyploid[5], *C. gracillimum* var. *byssoideum* is a cytologically polymorphic species, which has attained a higher level of polyploidy.

We are thankful to late Prof. T. Sreeramulu (Andhra University) for initiating us into these studies and one of us (M. S.) is thankful to CSIR for financial assistance.

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Waltair 530 003, *March* 28, 1977.

---

1. Umamaheswara Rao, M. and Sreeramulu, T., *Bot. J. Linn. Soc.*, 1970, 63, 23.
2. Dawson, E. Y., *Marine Red Algae of Pacific Mexico,* Univ. S. Calif. Press, Los Angeles, California, A. Hancock Pacific Expeditions, 1962, 26 (1), 56 and 57.
3. Dixon, P. S., "The Rhodophyceae," In : *The Chromosomes of the Algae*, Ed. M. B. E. Godward, Edward Arnold (P) Ltd., London, 1966, p. 168.
4. Magne, F., *Cah. Biol. Mar.*, 1964, 5, 461.
5. Rao, B. G. S. and Sundari, M., *Curr. Sci.*, 1977 (In press).

MEASUREMENT OF SHEATH COLOUR IN CITRONELLA—*CYMBOPOGON NARDUS* (L.) RENDLE

Taxonomic classification of citronella species has been carried out by several investigators and the two principal species in cultivation are *Cymbopogon winterianus* Jowitt and *Cymbopogon nardus* (L.) Rendle[1,2]. The latter is mainly cultivated in Sri Lanka[3]. Several selections of this species have been identified on the basis of their morphological characteristics[4]. Sheath colour was used as one of these characteristics as it could be measured by a quick quantitative technique. This measurement involves the use of Munsell colour charts (Munsell Color Company Inc., Baltimore, Maryland, U.S.A.). The three attributes of Munsell colour charts are hue, value and chroma. Using a mask to facilitate matching of surface colours it is possible to obtain quantitative data for differences in sheath colour among citronella selections.

TABLE I

Sheath colour dimensions for ten citronella strains grown in controlled environments at three day/night temperatures

Selection	15/10° C		27/21° C		32/27° C	
	Hue	Value/chroma	Hue	Value/chroma	Hue	Value/chroma
C_1	10·0*RP	3/4	7·5*Y	5/2	7·5*Y	5/4
C_2	10·0 RP	5/4	2·5 Y	5/2	7·5 Y	5/4
C_3	10·0 RP	2/4	2·5 Y	5/4	7·5 Y	5/4
C_4	10·0 RP	4/4	2·5 Y	5/4	7·5 Y	5/6
C_5	10·0 RP	5/2	2·5 Y	4/2	7·5 Y	6/4
C_6	10·0 RP	4/2	10·0 YR	3/2	7·5 Y	5/4
C_7	10·0 RP	6/2	7·5 Y	5/2	2·5 GY	6/4
C_8	10·0 RP	3/4	2·5 YR	4/2	7·5 Y	5/2
C_9	10·0 RP	4/2	2·5 Y	4/2	10·0 Y	5/4
C_{10}	10·0 RP	4/2	2·5 Y	4/2	10·0 Y	5/4

* Gradations of hue.

In order to test the consistency of this method an experiment was conducted under controlled environmental conditions using three day/night temperatures, 15/10, 27/21 and 32/27° C. Three plants each of ten selections of *Cymbopogon nardus* were grown at each temperature and evaluated for sheath colour after 90 days. The results are given in Table I. There was a temperature effect on the development of all three attributes of sheath colour; hue, chroma and value. Hue (spectral quality) changed from reddish purple (RP) to yellow (Y) or hues near yellow (YR, GY) as the temperature increased from 15/10 to 27/21 or 32/27° C. Value (lightness) of yellow colouration increased slightly with temperature between 27/21 and 32/27° C in six selections (C_5, C_6, C_7, C_8, C_9 and C_{10}) and was unaffected in the other four. All selections but two (C_3 and C_8) increased in chroma (intensity or strength) of yellow colouration with the temperature increase from 27/21 to 32/27° C.

Even though the sheath colour differences are consistent in the field and can be used as a quick method of identifying selections, the present data indicate that there is a temperature by selection interaction. Not only can the sheath colour vary with the growing temperature, but the temperature effect differs among selections. All selections had the same reddish-purple hue at 15/10° C but this hue varied among selections in both value and chroma. At 27/21° C there were four categories of hue with C_1 and C_7 more greenish yellow than C_2, C_3, C_4, C_5, C_9 and C_{10} which were brownish yellow. C_6 was somewhat more brownish and C_8 more reddish than the other selections. There were also differences among selections in value and chroma. At 32/27° C, C_1, C_2, C_3, C_4, C_5, C_6, and C_8 had a greenish yellow hue, C_9 and C_{10} a more greenish and C_7 an even more greenish hue. There were also variations in value and chroma. At 15/10° C the 10 selections could be separated into 7 groups based on value and/or chroma differences. At 27/21° C there were 7 groups separated by hue, value and/or chroma differences and at 32/27° C the corresponding number of such groups was 6. These relationships among sheath colours of these selections differed with temperatures. Therefore identification of a selection by sheath colour would not be satisfactory if the selections were grown at some other temperature such as might occur at a different location.

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---

1. Senaratne, J. E., *The Grasses of Ceylon, Peradeniya Manual*, 1956, 8, 193.
2. Jowitt, J. F., *Annals Royal Botanic Gardens*, Peradeniya, 1908, **49**, 185.
3. Wijesekera, R. O. B., Jayawardene, A. L. and Fonseka, B. D., *Phytochemistry*, 1973, **12**, 2697.
4. Iruthayathas, E. E. and Herath, H. M. W., *Faculty of Agric. Seminar Series*, 1975, **75**, 6.

A DOUBLE TELO TRISOMIC FOR THE SEVENTH CHROMOSOME IN PEARLMILLET

IN the progeny of a self pollinated Semidwarf trisomic of pearlmillet[1], which is trisomic for the 3rd chromosome, one out of 29 plants had a chromosome constitution of $2n = 13$ normal *plus* two small chromosomes. The small chromosomes were clearly unequal in length and at pachytene were identified as telocentrics. The short and the long telos corresponded to the short and long arms of the 7th chromosome of pearlmillet, which is the shortest and possesses a satellite on the short arm[2]. Only one such normal nucleolus organising chromosome was observed in the present material. This chromosome and the short telo were almost always directly associated with the nucleolus in PMC's at prophase I, but not the long telo. A plant of such a chromosome constitution is called a double telo trisomic according to the nomenclature of Kimber and Sears[3].

In pearlmillet, the normal 7th chromosome pair occurs usually as a rod bivalent at meiosis but also rarely as a ring bivalent or two univalents. In the present plant in PMC's at prophase I and metaphase I no pairing was observed between the telos. However, in 68% of the 25 cells examined at metaphase I, the long telo paired with the normal chromosome to form a heteromorphic bivalent. In 4% of the cells, a short telo paired with the normal chromosome to form a heteromorphic bivalent. In 16% of the cells the two telos were paired one on either end of the normal chromosomes to form a heteromorphic chain trivalent and in 12% of the cells the three chromosomes remained as univalents.

In an earlier report on double telo trisomics for a different chromosome of pearlmillet[4]' centromere misdivision of one submetacentric chromosome was considered to have given rise to two telocentrics. A similar mechanism is possible in the present case involving a 7th chromosome univalent in the trisomic parent.

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---

1. Manga, V., *Ph.D. Thesis*, Andhra University, 1972.
2. Pantulu, J. V., *Curr. Sci.*, 1958, **27**, 497.
3. Kimber, G. and Sears, E. R., *Proc. III Intern. Wheat Genet. Symp.*, Canberra, 1968, p. 466.
4. Narasinga Rao, P. S. R. L., Subba Rao, M. V. and Narayana Rao, I. *Experientia*, 1977, **33**, 308.

TOP NECROSIS VIRUS DISEASE OF PEA FROM INDIA—A NEW RECORD

THE survey of local fields of Agra during 1975–77 revealed an important necrosis virus disease of pea (*Pisum sativum* L.).

The infected plants exhibited withering of the top (top necrosis) and greyish-brown discolouration of leaves, petioles and stems. Internally the host tissues were highly necrosed. Severely infected pea plants die premature death, whereas plants with mild infection develop malformed pods with wrinkled or even abortive seeds. Under glass house conditions (25° ± 3° C), typical systemic symptoms of the disease were reproduced on the healthy pea plants but only with occasional top necrosis.

The traditional methods of graft (wedge and cleft grafts) and sap inoculations (using phosphate buffer of pH 7·0 and carborundum powder) gave 100% transmission. The virus was also found to be transmitted through seeds (upto 13·6%) and by nematode vector (*Trychodorus* sp.).

The physical properties were studied at room temperature (25° ± 3° C) using *Chenopodium ameranticolor* Coste and Regn as local lesion host. Its dilution end point lies near 1 : 10,000, the thermal death point at 64° C and longevity *in vitro* and *in vivo* for 5 and 15 days respectively.

The present virus disease is a new record from India as it has been found to differ from all other virus diseases reported on peas[1-3],

The auther is thankful to Dr. K. S. Bhargava, Professor and Head, Department of Botany, Kirukshetra University, for rendering valuable suggestions.

Virus Research Lab,, A. N. ROY.
Department of Botany, M. N. GUPTA.*
Agra College, Agra 282 002,
April 16, 1977.

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* Professor and Head, Department of Botany, Agra College, Agra.

1. Bos, L., *Neth. J. Pl. Path.*, 1970, **76**, 8.
2. Gibbs, A. J. and Harrison, B. D., *Ann. Appl. Biol.*, 1964, **31**, 154.
3. Smith, K. M., *Text Book of Plant Virus Diseases* J and A Churchill Co., 1972.

REVIEWS AND NOTICES OF BOOKS

Utilization of Neem and its Byproducts (A Monograph). Edited by C. M. Ketkar. (Khadi & Village Industries Commission, 1153 Ganeshkind Road, Shivajinagar, Pune, 411016), 1976, Pp. 150. Price: Not given.

The Monograph embodies the results of a detailed study of the utility of various products of the Neem Tree (*Azadirachta indica* Juss.). Comprehensive data is presented pertaining to the following aspects of study, in five chapters, together with an exhaustive list of references at the end of each chapter.

(i) Mineralization of Neem Cake, oil and extractive in combination with urea, and its comparison with other slow release fertilizers, nitrification inhibitors and coated fertilizers; inhibitory effect of Neem Cake and extractive on Nitrosomanas and Nitrobactor (Chapter 1).
(ii) Effect of Neem Cake blended urea on the yield of paddy, wheat, maize, sorghum, sugarcane, potato and tobacco, under different climatic conditions of India (Chapter 2).
(iii) Utility of Neem Kernel and Neem Oil in preventing infestation of crops and stored grains, by pests (Chapter 3).
(iv) Efficacy of Neem Cake and extractive against nematodes attacking potato, tobacco, betel vine, tomato, okra, cabbage and cauliflower (Chapter 4).
(v) Neem Cake as a livestock feed, and the medicinal uses of Neem leaves and oil (Chapter 5).

The Monograph is an example of how the potential value of the minor seeds of tree origin could, by systematic and coordinated study, be brought to light. This serves as a pointer to the necessity of conducting detailed investigations on other minor seeds of tree origin.

The get-up and print of the Monograph are satisfactory. The typographical errors appearing in a number of pages could have been avoided with a little caution.

The Monograph should prove a useful addition for the Libraries of all Agricultural and Veterinary Colleges.

K. PARTHASARATHI.

Mathematical Methods for Physics. By H. W. Wyld. (W. A. Benjamin, Inc., Reading, Massachusetts, 01867), 1976. Pp. 628. Price $26.50.

'*Mathematical Methods for Physics* by H. W. Wyld (W. A. Benjamin Inc., 1976) is an eminently readable book. While the basic material is no different from that in many other texts available in most science libraries, it is the informal tone of presentation of the otherwise fairly regorous mathematical treatment that appeals to the reader. The reader in this case is most probably a graduate student in physics. However, those engaged in physics teaching will also find the book equally valuable. The book covers most of the topics in mathematics that one is likely to encounter in classical physics. Thus, partial differential equations, special functions and Green's functions have been treated in detail. The author obviously has classical electromagnetism at the back of his mind. But, by far the most illuminating chapter is the one on dispersion relations, namely Chapter 12. Here the author has successfully brought out the tremendous power of these relations without invoking any highbrow stuff. In fact anyone with an average knowledge of complex analysis can go ahead with this chapter and assimilate the ideas as well as the technique. The same is true of Chapter 14 on Integral transforms in the complex plane. There is a notable omission however. Matrix analysis has been given a complete go by. But, obviously, the author is not aiming at completeness of any sort. On the whole the book is highly recommended.

N. KUMAR.

Programming in Fortran IV. By K. D. Sharma. (Affiliated East West Press Private Limited, India), 1976. Pp. 258 + 120 illustrations. Price Rs. 25·00.

The book is organised in 7 chapters followed by 6 appendices.

The first chapter introduces the basic principles of digital computer operation, functional aspects of the various units like input/Output media, memory, and arithmetic and control units, and the role of programming languages are emphasized. In the second chapter the principles of programming, construction and the use of flow charts and broad classification of FORTRAN statements are discussed. Fortran language fundamentals and methodology of constructing simple programs are explained in the 3rd chapter. Logical decision making and transfer of control facilities available in Fortran are discussed in the 4th chapter. In the 5th chapter representation of subscripted variables in computer, and the role of DO loops are exhaustively covered. The 6th chapter deals with function and subroutines. Further facilities available in FORTRAN, which will help the programmer in handling sophisticated problems with specific relevance to precision, complex arithmetic and advanced input/output operations, etc., are explained in the 7th Chapter.

But for a few typographical errors, and lack of reference of current titles like integrated circuits, multiprogramming, memory overlay, virtual storage, structured programming concepts, etc., the subject has been very nicely developed and covered in sufficient depth. The role of various Fortran statements are lucidly explained, with notes of caution, for programmes in their use. Ample number of illustrative examples, flow charts and program listings, encompassing wide application areas in science and engineering are provided to help in the grasp of the basic concepts; also useful tips for developing efficient programs and to reduce the execution times are given at appropriate stages throughout the text. A number of good problems are listed at the end of chapters for the students to try and the solutions of these problems have also been provided in the end for ready cross checking. Tables, explicitly indicating comparison of salient features and constraints in FORTRANS of various digital computers available in the country are given for the guidance of students in the use of the respective machines. Information which is likely to be frequently referenced has also been systematically documented in the appendices.

This book adequately fulfils its objectives. It could be fruitfully used in teaching a first course for the undergraduates and also it is recommended to all those who wish to learn Fortran through self-study.

A. A. Shamim.

SPECIAL TRAINING PROGRAMME IN WILD LIFE BIOLOGY, SPONSROED BY U.G.C., NEW DELHI,

JANUARY 1-JUNE 30, 1978

The aim of this special training programme is to acquaint College and University teachers and Ph.D. students in biology with the fascinating world of wild life of India. The programme will involve three months of intensive training in field at the famous tiger project reserve of Bandipur, followed by three months of project work at any wild life sanctuary of the participant's choice. It is hoped that following this training, many of the participants will establish schools of training and research in wild life biology at their respective institutions.

The three month training programme at Bandipur National Park will involve course work in the areas of ecology, behaviour, sociobiology, management of biological resources and resource management. The lectures will be closely supplemented by outdoor practical sessions. And all this will be in the lovely setting of wild life at Bandipur.

The faculty will include both professional biologists from Indian Universities, and forest officers with an intimate knowledge of the wild life.

Participants

About fifteen. The programme is open to all College and University teachers of biology, as well as students already registered for a Ph.D. in biology at any Indian University. Exceptional candidates who do not belong to these categories will also be considered if space is available.

Applications

The last date for applying is 30th September 1977. There are no application forms. For details please write to Dr. Madhav Gadgil, Centre for Theoretical Studies, Indian Institute of Science, Bangalore 560 012. Please also name two referees who will write directly to Dr. Madhav Gadgil about you.

Financial Assistance

The participants will receive a fellowship of Rs. 400/- per month throughout the six month period. They will also be provided with the necessary funds for contingent expenditure during the project work. Any travel involved will also be paid for. Please write to the Education Officer, University Grants Commission, Bahadurshah Zafar Marg, New Delhi 110001, for any assistance regarding obtaining leave from your parent institution and other administrative problems.

6518/77. Published by S. R. S. Sastry, Manager, Current Science Association, Bangalore-560006
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TITANIUM(IV) COMPLEXES OF BENZOYL HYDRAZONES

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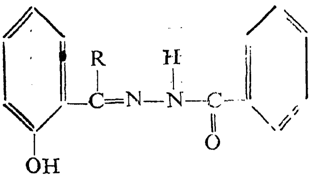
Department of Chemistry, Meerut College, Meerut 250001

ABSTRACT

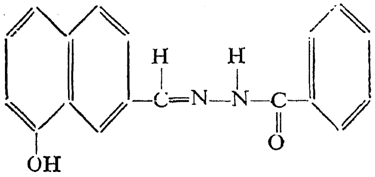
Titanium(IV) complexes of benzoyl hydrazones derived from benzhydrazide and salicylaldehyde (BSH), *o*-hydroxyacetophenone (BAH), *o*-hydroxypropiophenone (BPH), *o*-hydroxybutyrophenone (BBH) and 2-hydroxynaphthaldehyde (BNH) have been described. These complexes have been characterised by elemental analyses, and by conductance, magnetic, infrared and electronic spectral measurements. The complexes have trans-octahedral stereochemistry as indicated by ν (M–N) and ν (M–O) vibrations in far infrared region.

INTRODUCTION

INTEREST in the coordination chemistry of titanium(IV) ion continues to increase on account of its capability to form coordination compounds having unusual coordination numbers[1-3]. A number of such studies are available with Schiff bases[4-8]. In this paper, we report the synthesis and characterisation of titanium(IV) compounds with benzoyl hydrazones whose structures are given below :



R = H, CH_3, C_2H_5, C_3H_7



EXPERIMENTAL

All the reagents used were of Analar grade. The ligands were synthesised by the method of Struve[9]. They all gave satisfactory analyses for C, H, N and their purity was further checked by TLC.

Synthesis of Complexes

The titanium(IV) chloride was (0·001 mole) diluted to 50 ml with absolute alcohol. The addition of 0·002 mole of the ligand in 50 ml acetone to the above solution instantaneously gave a bright red precipitate. It was digested for about 15 minutes at 70° C, cooled, filtered and washed several times with acetone. The product was recrystallised from chloroform and dried in vacuum.

The conductance, magnetic moment, i.r. and electronic spectral measurements were made as reported previously[10]. Titanium was estimated gravimetrically as titanium dioxide. Analytical data are given in Table I.

RESULTS AND DISCUSSION

The analytical data reveal 1 : 2, metal : ligand stoichiometry and the complexes are represented by general formula [Ti(Lig)$_2$]. They are soluble in acetone, alcohol, chloroform, DMF and DMSO. The molecular conductance measurements in DMF (25° C) indicate that these complexes are non-electrolytes. This formulation is further supported by infrared spectra.

The i.r. spectra of the ligands show a strong band between 3200–3250 cm^{-1} which may be assigned to ν(OH) vibrations involving hydrogen bonding. The intramolecular hydrogen bonding is also indicated by the presence of weak broad band at 2700–2775 cm^{-1} in the free ligands[5,11]. The bands at 3000–3050 cm^{-1} are assigned to ν(NH).

Strong bands in the region 1660–1675, 1615–1625 and 1600 cm^{-1} in the free ligand may be assigned to amide I (due to ν C=O), ν(C=N) (azomethine) and phenyl ring vibrations[5,12], respectively. The bands around 1515, 1250, 650 and 495 cm^{-1} may be assigned to amide II (νCN+δNH), amide III (ν CN), amide IV (C=O out of plane deformation) vibrations, and amide VI (C=O in plane deformation, respectively, indicating that the ligands are persent in keto form[12-14].

Dramatic changes are found in the spectra of the complexes which show no band characteristic of amide and amino group suggesting that the ligands are not bound in keto form. The ν(C=N) band shows a downward shift (15–20 cm^{-1}) with slight loss of intensity. This downward shift indicates the coordination of azomethine nitrogen. A new band appearing at ~ 1598 cm^{-1} seems to have its origin in the stretching vibration mode of the conjugate $\left(\right\rangle C=N-N=C\left\langle\right)$ grouping analogous to that of azine[15], suggesting the participation of enolic oxygen. The disappearance of bands 3200–

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* For correspondence.

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TABLE I

Analytical Data

Compound	Colour	Percentage composition							
		Calcd.				Foind			
		C	H	N	Ti	C	H	N	Ti
Ti $(BSH)_2$	Bright red	63·89	4·21	10·61	9·10	64·12	4·25	10·55	9·02
Ti $(BAH)_2$	Light brown	64·98	4·73	10·10	8·64	64·80	4·75	10·01	8·75
Ti $(BPH)_2$	Light brown	65·98	5·19	9·62	8·22	65·50	5·22	9·70	8·30
Ti $(BBH)_2$	Brown	66·88	5·61	9·17	7·84	67·02	5·55	9·10	7·90
Ti $(BNH)_2$	Yellowish red	69·16	4·18	8·94	7·64	69·01	4·10	8·96	7·60

TABLE II

Important infrared bands (in cm^{-1}) and their assignments

Compound	Amide I	ν(C = N)	( )C = N – N = C( )	ν(Ti – N)	ν(Ti – O) Phenolic	ν(Ti – O) enolic
BSH	1660 s	1620 s	..	..	..	..
BAH	1675 s	1615 s	..	..	..	..
BPH	1665 s	1625 s	..	..	..	..
BBH	1670 s	1625 s	..	..	..	..
BNH	1665 s	1602 s	..	..	..	..
Ti $(BSH)_2$	..	1602 s	1595 s	540 s	460 w	375 m
Ti $(BAH)_2$	..	1600 s	1596 m	540 m	458 w	368 m
Ti $(BPH)_2$	..	1607 m	1600 m	532 s	461 m	372 m
Ti $(BBH)_2$	..	1609 s	1600 s	535 s	460 w	370 w
Ti $(BNH)_2$	..	1602 s	1595 s	530 m	460 m	368 m

3250 and 2700–2775 cm^{-1} supports the involvement of phenolic oxygen in coordination through deprotonation[11]. From these changes it appears that these ligands are terdentate with coordination taking place through phenolic and enolic oxygen (with deprotonation in both) and azomethine nitrogen. This is further supported by the appearance of a strong band 530–540 and bands around 445–450 and 400–405 cm^{-1} assignable to ν(Ti–N), ν(Ti–O) (phenolic) and ν(Ti–O) (enolic) vibrations[6,16], respectively. It has been observed that titanyl complexes[17] exhibit a strong band $\sim$1090 cm^{-1} assignable to ν(Ti=O). However, in the present complexes no such band has been observed proving that these complexes do not contain any Ti=O species.

Titanium(IV) is a d° system and thus no conclusions regarding its stereochemistry can be drawn from its magnetic and electronic spectral studies. The electronic spectra of all these compleyes show a single band in the region 20000–22500 cm^{-1} which may be due to charge-transfer[18]. Magnetic measurements indicate that they are diamagnetic in nature. However, in far infrared region ν(M–N) and ν(M–X) (X = Cl, Br, etc.), have been of specific use in assigning the stereochemistry of close *d*-shell systems[19]. It is known that *cis*-complexes give rise to two metal-ligand (M–N) vibrations while only one such vibration is observed for *trans*-complexes[20]. In the present case, only one ν(M–N) vibration has been observed in the region (530–540 cm^{-1}) assigned to *trans* six-coordinate octahedral complexes. The metal-oxygen vibrations known to be sensitive to the coordination number and oxidation state[21] of the central metal ion have consistently been observed around the same frequency (405, 450 cm^{-1}) indicating that these complexes are *trans*- octahedral and the metal atom has the same oxidation state and the same coordination number in these complexes.

ACKNOWLEDGEMENT

The authors are thankful to the U.G.C. and C.S.I.R. New Delhi, for financial support.

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1. Bhatt, A. N., Fay, R. C., Lewis, D. F., Lindmark, A. F. and Strauss, S. H., *Inorg. Chem.*, 1974, 13, 886.

2. Alyea, E. C. and Merrel, P. H., *Inorg. Nucl. Chem. Letters,* 1973, 9, 69.
3. —, Rameswamy, B. S., Bhatt, A. N. and Fay, R. C., *Ibid.,* 1973, 9, 399.
4. —, Malek, A. and Marrel, P. H., *J. Coord. Chem.,* 1974, 4, 55.
5. — and —, *Can. J. Chem.,* 1975, 53, 939.
6. Birader, N. S., Mahle, V. B. and Kulkarni, V. H., *Inorg. Chim. Acta,* 1972, 7, 267.
7. Bradley, D. C., Hursthouse, M. B. and Rendall, I. F., *Chem. Commun.,* 1969, p. 672.
8. Gilli, G., Cruickshank, D. W. J., Beddoes, R. L. and Mills, O. S., *Acta. Cryst.,* 1972. 28 B, 1889.
9. Struve, G., *Pract. Chem.,* 1894, (2), 50, 295.
10. Sahni, S. K., Gupta, S. P., Sangal, S. K. and Rana, V. B., *J. Inorg. Nucl. Chem.,* (In press).
11. Nakamoto, K., *Infrared Spectra of Inorganic and Coordination Compounds,* John Wiley and Sons, New York, 1970.
12. Nonoyama, M. and Yamasaki, K., *Inorg. Chim. Acta,* 1969, 3, 385.
13. Miyazawa, T., Shimanouchi, T. and Mizushima, S., *J. Chem. Phys.,* 1958, 29, 611.
14. Nonoyama, M., Tomita, S. and Yamasaki, K., *Inorg. Chim. Acta,* 1975, 12, 33.
15. El Sayed, L. and Iskander, M. F., *J. Inorg. Nucl. Chem.,* 1970, 33, 435.
16. Birader, N. B. S., Mahle, V. B. and Kulkarni, V. H., *Inorg. Nucl. Chem. Letters,* 1972, 8, 997.
17. Barrachough, C. C., Lewis, J. and Nyholm, R. S., *J. Chem. Soc.,* 1959, p. 3552.
18. Dunn, T. M., Nyholm, R. S. and Yamada, S., *Ibid.,* 1962, p. 1564.
19. Clark, R. J. H., *Spectrochim. Acta,* 1965, 21, 955.
20. Jain, S. C. and Riuest, R., *J. Inorg. Nucl. Chem.,* 1970, 32, 1117.
21. Adams, D. M., *Metal-Ligand and Related Vibrations,* E. Arnold, London, 1967.

STUDIES ON THE INFLUENCE OF DRAINAGE AS A CULTURAL PRACTICE IN INCREASING RICE YIELDS

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AND

M. R. BISWAS

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ABSTRACT

Field experiments were conducted to find out the effect of drainage on the growth and yield of rice, grown continuously under a standing water of 5 inches. Plots having drainage provision indicated 28 per cent and 49 per cent increase in rice yield for the varieties CHANDINA and IRATOM-24, respectively, over the rice yield in plots without drainage provision. Analysis of standing water in the field and drain water after an application of 23 kg of nitrogen per hectare on standing water indicated that the applied N is mostly absorbed and retained by soil possibly by diffusion.

INTRODUCTION

It is well known that highest yields of rice (5–6 tons per hectare) are obtained in Japan and Taiwan and this is usually attributed to the heavy fertilisation and improved cultural practices[1]. These practices when applied, however, did not increase the yields significantly in the developing countries, and the highest yield recorded with high yielding varieties was of the order of three tons per hectare. Both Japan and Taiwan have an additional cultural practice, *viz.*, provision of separate drainage channel parallel to irrigation channel in the rice fields[2-7]. Drinage through the root zone has its own significance in a crop grown with standing water and may contribute significantly to grain yield[3-6].

Experiments both on the field and in the laboratory are, therefore, conducted at the Institute of Nuclear Agriculture with a view to understanding the role of drainage in increasing the rice yield and the effectiveness of broadcasting of fertilizer N on standing water surface in rice fields.

EXPERIMENTAL

A statistically laidout field experiment with and without provision of drainage was initiated on a newly cultivated INA experimental field (Brahmaputra alluvial soils). Treatment A (irrigation with no drainage) and Treatment B (irrigation with drainage) were laid in pair of plots, there being 4 pairs for each treatment. For the purpose of performance study, 2 pairs of each treatment were randomly chosen. Irrigation was provided as to keep a level of 5″ standing water in all the plots. Four weeks old rice seedlings were transplanted uniformly in all the fields. Fertilizer N, P and K @ 90 kg of N, 90 kg of P_2O_5 and 70 kg of K_2O per hectare were applied. Nitrogen, however, was given in three splits. The drainage channels were two feet deep and were good enough to drainwater through the rice root zone in the fields.

RESULTS AND DISCUSSION

The mean grain yields obtained in the experimental plots are recorded in Table I.

TABLE I

Effect of drainage on rice yields (Mean of two pair plots in Kg/ha)

Treatments	CHANDINA (Aman season 1975)		IRATOM-24 (Boro season, 1976)	
	Mean yields	Percentage increase in yields	Mean yields	Percentage increase in yield
Field with no drainage	2935	28*	1849	49*
Field with drainage	3752		2790	

* Significant at 5% level.

All other conditions remaining constant, field with provision of drainage increased the Chandina yield by 28% significant at 5% level. A second crop of IRATON grown on the same layout showed 49% increase in grain yields in the field provided with drainage (significant at 5% level). This increase in grain yield conforms to the finding of the experiment conducted in Taiwan, Philippine, Thailand and Japan[1,2] where two fold increase in the yield has been recorded from fields provided with drainage. This increase is spectacular in contrast to poor yields commonly obtained by farmers after providing all the inputs thought to give the maximum yields.

Twenty-three kg. of nitrogen was applied as a second dose on the standing water surface.

Nitrogen content of water collected from field plots and drains every 24 hours after the application of second dose of urea is shown in Fig. 1. Concentration of nitrogen in standing water from the field plots without drainage provision was found to be slightly higher than the nitrogen content of water in the field plots with drainage provision. Nitrogen concentration of standing water in both drained and non-drained plots was found to be maximum at 24 hours after the application of urea but the nitrogen content of water collected at 48 and 72 hours after application was found to decrease considerably (curves A and B). Concentration of nitrogen in standing water though increases rapidly in the first 24 hours, yet it decreases exponentially with time and reaches the original level of concentration in 72 hours possibly by the diffusion of the nutrient into the soil. The nitrogen concentration of drain water, however, increases upto 48 hours (curve C). The nitrogen concentration of the drain water was too low in comparison with that of the standing water of field plots. The delayed increase in nitrogen concentration may arise from the time lag in the process of infiltration due to tortuosity likely to be encountered in the rice field.

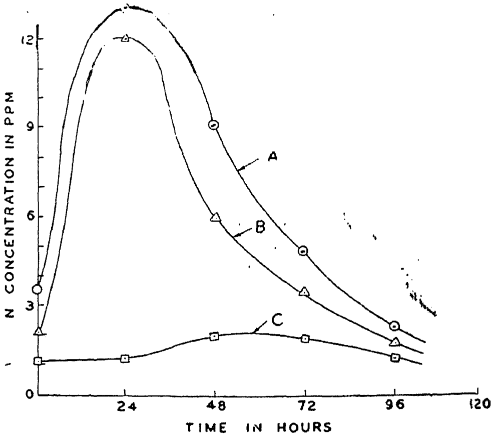

FIG. 1. Concentration of Nitrogen in standing water and drain water of rice fields.

A, Standing water of field plots without drainage; B, Standing water of field plots with drainage; C, Drain water.

With a view to understanding the mode of disposal of nitrogen within the first 72 hours after application on the water surface, a laboratory experiment simulating the field conditions was conducted in pots in duplicate keeping the infiltration rate same as in normal rice soils, *viz.*, at 2 mm level per day. The results are given in Fig. 2. The rapid fall in the nitrogen concentration of the standing water in pots between 24 and 72 hours

(2A) at [illegible] formed [illegible] water [illegible] centration [illegible] nitrogen [illegible] a indicated [illegible] B [illegible]

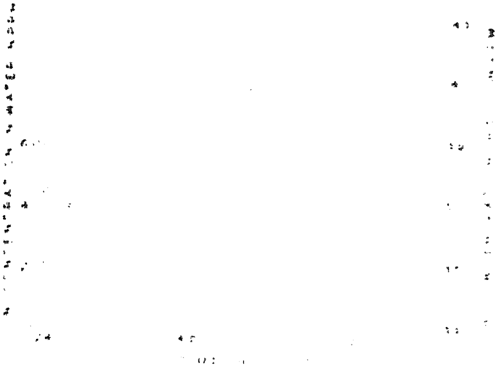

FIG. 2 Concentration of Nitrogen in standing water, Soil and [illegible] experiment A, Standing water, B, Soil, C, [illegible]

The results [illegible] experiment clearly indicated the significant increase of grain yields in rice plots provided with drainage and also confirmed that the practice of applying nitrogen fertilizer on standing water [illegible] ing on the wet soil [illegible] seems to have the capacity to absorb [illegible] nitrogen applied.

Brahmaputra flood plain soil [illegible] infiltration rates much higher than the [illegible] ing heavy soil of Bangladesh [illegible] The beneficial effects of drainage [illegible] more prominent on the traditional [illegible] growing soil. The experiment is, therefore, being repeated [illegible] at other research centres [illegible] representing Brahmaputra [illegible] flood plain soil and Ganges [illegible] flood plain soils with impeded drainage.

Further investigations are in progress to determine deletion removal [illegible] from the rice plots [illegible] under the stress of plant uptake and the loss [illegible] of irrigation water [illegible]. The influence of additional [illegible] with the water moving laterally [illegible] on the root and consequent [illegible] under study.

ACKNOWLEDGEMENTS

The project was largely initiated by Dr C. Dakshinamurti, IAEA Adviser and his active help [illegible] the project is gratefully acknowledged.

The authors also acknowledge the unhindered support of Dr M. M. Mia, Director, INA, in the [illegible] of the project and Dr A. [illegible] Ali of [illegible], for help in statistical design of the experiment.

1 Dakshinamurti, C., Michael, A. M., Mohan, S., and Pandey, S. L., *Report on the Visit to Thailand and Philippines*, Water Technology Centre, IARI, New Delhi, 1973, p. 1.

2 Fukuda, H., "The development of Japanese irrigated agriculture (Resource paper)," *Regional Workshop on Irrigation Water Management*, Asian Development Bank, 1972, p. 199.

3 IRRI, "The mineral nutrition of rice plant," *Proceedings of a Symposium at IRRI*, The John Hopkin Press, Baltimore, Maryland, 1964, p. 494.

4 Kramer, P. J., *Plant and Soil Water Relationships, A Modern Synthesis*, McGraw Hill Book Co., N.Y., 1969, p. 100.

5 USDA, "Agriculture Research Services," *Rice in the United States: Varieties and Production*, Agriculture Hand Book No. 289, 1966.

6 Van't Woudt, B. D. and Hagan, R. M., "Crop responses at excessively high soil moisture levels," *Drainage of Agricultural Land*, edited by J. W. Luthin, American Society of Agronomy, Madison, Wisconsin, 1957, p. 514.

7 Wang, Y. T., "Development impact and Manganese of Irrigation in Taiwan (Resource paper)," *Regional Workshop on Irrigation Water Management*, Asian Development Bank, 1973, p. 211.

CHEMOTAXONOMICAL STUDIES ON CAPPARIDACEAE—CLEOMACEAE

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ABSTRACT

12 members belonging to Capparidaceae and Cleomaceae have been analysed for flavonoids and phenolic acids. The distribution pattern of these compounds among the members studied indicate that the two families are quite distinct chemically. Hutchinson's separation of these two families, therefore, is supported. The chemical affinity of Cleomaceae to Brassicaceae has been clearly brought out.

INTRODUCTION

CAPPARIDACEAE (Capparaceae), a comparatively small family, is morphologically peculiar in the presence of a gynophore and uniloculate ovary with parietal placentation. This family comprises members of varying habits which ranges from herbs like *Cleome* to trees like *Crateva.* Based on the habit and other morphological characters it has been segregated into two groups Cleomoideae and Capparideae. Cleomoideae include all the herbaceous members which are having dehiscent fruits with a replum, *i.e.,* a frame like placenta from which the valves fall away in dehiscence and Capparideae of woody perennials having indehiscent fruits. These two segregates have been considered as two natural groups within the family.

Hutchinson in his separation of woody and herbaceous plants to Lignosae and Herbaceae, retained the herbaceous members (Cleomoideae) in the Lignosae, as a subfamily Cleomoideae in Capparidaceae[4]. But after a more intensive study "genus by genus and species by species, in the Kew Herbarium," he came to the conclusion that the family as it then existed consists of two distinct groups which are not really phylogenetically related. In his opinion "the true capparids with Capparis as its generic type are woody plants and have indehiscent fruits without a replum and are closely related to Flacourtiaceae (Bixales), whilst the subfamily Cleomoideae, type genus Cleome, are related to Brassicaceae (Cruciferae), all being with dehiscent fruits provided with a replum." In his later work[5] he raises these two subfamilies to the family status and separates them to very distant groups. Capparidaceae is grouped, in order Capparidales of Lignosae along with Moringaceae and Cleomaceae in Cruciales of Herbaceae with Oxystylidaceae and Brassicaceae.

Not much work has been done on the chemistry of this group. The earlier chemical reports[2,9,11,12] are quite insufficient for a chemotaxonomical treatment of these families. Recently Das and Rao[1] have attempted a phytochemical phylogenetic study of the groups using phenolic compounds as markers. In the present work, 12 members com under 5 genera have been screened for leaf phenoli Of these 5 belong to Cleomaceae and the rest Capparidaceae.

MATERIALS AND METHODS

All the plants were collected fresh. Voucl specimens have been deposited in the Herbariu Department of Botany, The M.S. University Baroda, India.

Standard procedures[3,6,8,13] were adopted the extraction, isolation and identification various phenols, present in leaves of these plant

RESULTS AND DISCUSSION

The distribution of various flavonoids a phenolic acids in different members of Capparic ceae—Cleomaceae is presented in Tables I and I

Flavonols are found to be present in 10 pla screened. They are quercetin, rhamnetin, i rhamnetin, kaempferol and myricetin. Querce is present in at least 50% of the species examin isorhamnetin in more than 40% and rhamne about 15%. Kaempferol and myricetin are prese in one member each. Of these quercetin in *Cada indica,* isorhamnetin in *Cleome chelidonii* a myricetin in *Crateva adansonii* are in traces.

Glycoflavones could be located in 3 plants on and they are isovitexin from *Crateva adanso* and isoorientin from *Cadaba indica* and *Cappa sepiaria.* Only *Crateva* gives a strong positive t for leucoanthocyanins, but *Capparis sepiaria* gi a weak positive test for them.

Out of the various phenolic acids identifi *p*-hydroxybenzoic acid is present in all the pla screened and vanillic acid in all but one. Syrin and ferulic acids are present in at least 75% the plants.

The chemical differences between the two famil can be summarised as follows :

1. Quercetin is more frequent in Cleomace Here, out of the 5 plants, 4 contain quercet and out of the 7 plants of Capparidace only 2 contain this compound. (The tra amounts of quercetin in *Cadaba* is to noted).

TABLE I

The distribution of flavonoids in Capparidaceae—Cleomaceae

	1	2	3	4	5	6	7
CAPPARIDACEAE							
Cadaba indica Lamk.	+	·	·	·	·	+	·
Capparis decidua Edgew.	·	+	·	·	·	·	·
C. grandis Linn. f.	+	·	+	·	·	·	·
C. sepiaria Linn.	·	·	+	·	·	+	+
C. zeylanica Linn.	·	·	·	·	·	·	·
Crateva adansonii DC.	·	·	·	·	+	+	+
Maerua oblongifolia A. Rich.	·	·	·	·	·	·	·
CLEOMACEAE							
Cleome chelidonii Linn. f.	·	+	·	·	·	·	·
C. cynandra Linn.	+	+	·	·	·	·	·
C. monophylla Linn.	+	·	·	+	·	·	·
C. simplicifolia Hf. ahd T.	+	+	·	·	·	·	·
C. viscosa Linn.	+	+	·	·	·	·	·

1. Quercetin, 2. Isorhamnetin, 3. Rhamnetin, 4. Kaempferol, 5. Myricetain, 6. Glycoflavones, 7. Leucoanthocyanins.

TABLE II

The distribution of Phenolic acids in Capparidaceae—Cleomaceae

	1	2	3	4	5	6	7	8	9	10	11	12
CAPPARIDACEAE												
Cadaba indica Lamk.	+	·	·	+	+	·	·	+	·	·	·	·
Capparis decidua Edgew.	+	+	+	+	+	+	+	·	+	·	+	+
C. grandis Linn. f.	+	+	+	+	+	+	+	·	+	·	+	·
C. sepiaria Linn.	+	+	+	+	+	+	+	·	+	·	+	+
C. zeylanica Linn.	+	·	·	+	+	·	·	·	+	·	+	·
Crateva adansonii DC.	+	·	·	·	+	·	·	+	+	+	·	·
Maeura oblongifolia A. Rich.	+	·	+	·	·	·	·	·	·	·	·	·
CLEOMACEAE												
Cleome chelidonii Linn. f.	+	·	·	+	+	·	·	·	·	+	·	+
C. gynandra Linn.	+	·	·	+	+	·	·	+	+	+	·	·
C. monophylla Linn.	+	+	·	·	+	·	·	·	+	+	·	·
C. simplicifolia Hf. and T.	+	·	·	+	+	+	·	·	+	·	+	·
C. viscosa Linn.	+	·	·	+	+	·	·	·	+	+	·	·

1. *p*-Hydroxybenzoic acid, 2. Protocatechuic acid, 3. Salicylic acid, 4. Syringic acid, 5. Vanillic acid 6. Genetisic acid, 7. 2-Hydroxy 6-methoxy benzoic acid, 8. 2-Hydroxy 4-methoxy benzoic acid, 9. Ferulic acid 10. Sinapic acid, 11. *p*-Coumaric acid, 12. *o*-Coumaric acid.

2. Isorhamnetin is present in 4/5 plants of Cleomaceae and 1/7 of Capparidaceae,

3. Rhamnetin, leucoanthocyanins and glycoflavones are present only in Capparidaceae.

4. Salicylic and 2-hydroxy, 6-methoxy benzoic acids are confined to Capparidaceae and also there is more frequency of protocatechuic, *p*-coumaric, *o*-coumaric and gentisic acids here. Whereas sinapic acid is more frequent in Cleomaceae.

The distribution of flavonoids and phenolic acids clearly define the two famalies. Not only the higher frequency, but also the higher concentration of flavonols in Cleomaceae is noteworthy. The phenolic acids also show clear separation of the groups. All these facts point to the justification of splitting the old family Capparidaceae to Capparidaceae sensulato and Cleomaceae, on morphological grounds by Hutchinson[4]. The distribution of alkaloids is also in agreement with this. Form Capparidaceae sensulato stachydrines and Capparis base are reported whereas from Cleamaceae brassicines[10], emphasizing the chemical distinctiveness of both.

These two families can be considered as natural groups. Cleamaceae have a high frequency of isorhamnetin and sinapic acid. Capparidaceae have glycoflavones, salicylic acid and 2-hydroxy, 6-methoxy benzoic acid confined to them and a comparatively higher frequency of some other phenolic acids among the members. This is also in close agreement with the view that these two taxa are phylogenetically not very closely related. The overlapping chemical characters can be looked upon as mere cases of parallel evolution. However, a clear picture will emerge only after a detailed analysis of a large number of taxa belonging to these families.

The presence of isorhamnetin in most of the members of Cleomaceae seems to be phylogenetically importent. Isorhamnetin is said to be a characteristic compound of the Brassicaceae[2] (Cruciferae), which indicates a closer chemical affinity between Cleomaceae and Cruciferae. The alkaloids. Brassicines reportel from *Brassica, Lepidium* and *Sinapis* are also present in *Cleome* and *Gynandropsis*[10]. This is also in support of Hutchinson's grouping of these two families in order Cruciales[5] on morphological grounds. Das and Rao[1], although recognising the similarity between the herbaceous *Cleome* and the Brassicaceae, derive the latter from the arborescent Capparids. Based on the chemical data regarding isothiocynate-producing glucosides, such a grouping has also not been favoured and Capparidaceae (including Cleomace have been grouped with other thioglucoside c taining families like Resedaceae, Moringaceae Cruciferae under order Rhoeadales[7]. The th glucosides have been detected in a number unrelated families. Their occurrence has b found to be sporadic and unreliable even in oth wise very related species, thereby reducing c siderably the value of this chemical marker. Acco ing to Kjaer[7] "Many more species must be exami before we can properly evaluate the more detai distribution patterns of the isothiocyan producing glucosides."

Taking into consideration all the known chemi data, more weightage has been given to the va of phenolics and alkaloids as markers, at least f the present.

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1. Das, V. S. R. and Rao, K. N., *Die Naturwi schaften Jahrgang*, 1975, 62, Heft, 12, 577.
2. Gupta, R. K., Chandra, S. and Mahade V., *Ind. J. Pharm.*, 1968, 30 (5), 127.
3. Harborne, J. B., *Comparative Biochemist Flavonoids*, (ed.), Academic Press, Lond 1967, p. 37.
4. Hutchinson, J., *The Families of Flower Plants*, Oxford Univ. Press, New Yo 1959.
5. —, *Evolution and Phylogeny of Flower Plants*, Academic Press, London, 1969.
6. Ibrahim, R. K. and Towers, G. H. N., *Ar Biochem. Biophys.*, 1960, 87, 125.
7. Kjaer, A., In : *Comparative Phytochemis* (ed., T. Ewain), Academic Press, Lond 1966, p. 193.
8. Mabry, T. J., Markham, K. R. and Thom M. B., *The Systematic Identification Flavonoids*, Springer Verlag, New Yo 1971, p. 35.
9. Pagani, F. and Romussi, G., *Farmaco. Ed. S* 1970, 25 (10), 727.
10. Raffauf, R., *A Hand Book of Alkaloids a Alkaloid-containing Plants*, Wiley-Inte Science, New York, 1970.
11. Rochleder, F. and Hlaziwetz, H., *Liebigs Ann* 1852, 82, 196.
12. Subramanian, S. S., Nair, A. G. R. an Nagarajan, S., *Curr. Sci.*, 1965, 34 (8 246.
13. Sreeramulu, N. and Rao, I. M., *J. Ind. Bo Soc.*, 1971, 40, 342.

PRELIMINARY STUDIES ON THE EFFECTS OF CADMIUM CHLORIDE ON THE MEIOTIC CHROMOSOMES

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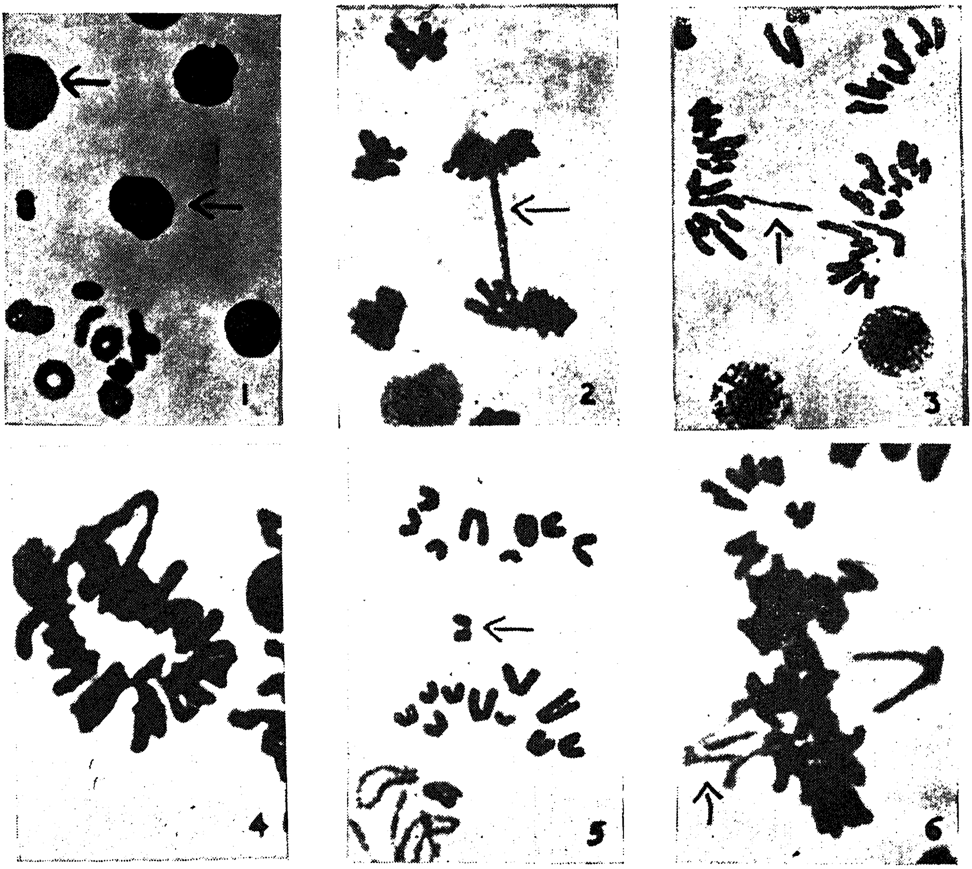
EXPERIMENTALLY it has been convincingly shown by many workers that the dreadful pollutant cadmium and its compounds cause harmful effects in mammals and man. Eventhough a lot of work has been done on the histological, histochemical, histophysiological, pathological and toxicological aspects of cadmium on mammalian tissues[1], very little work has been done on the effects of cadmium salts on the chromosomes. So, the present study deals with the effects of Cadimum chloride on male meiotic chromosomes of the grasshopper *Poekilocerus pictus* (Acrididae: Orthoptera).

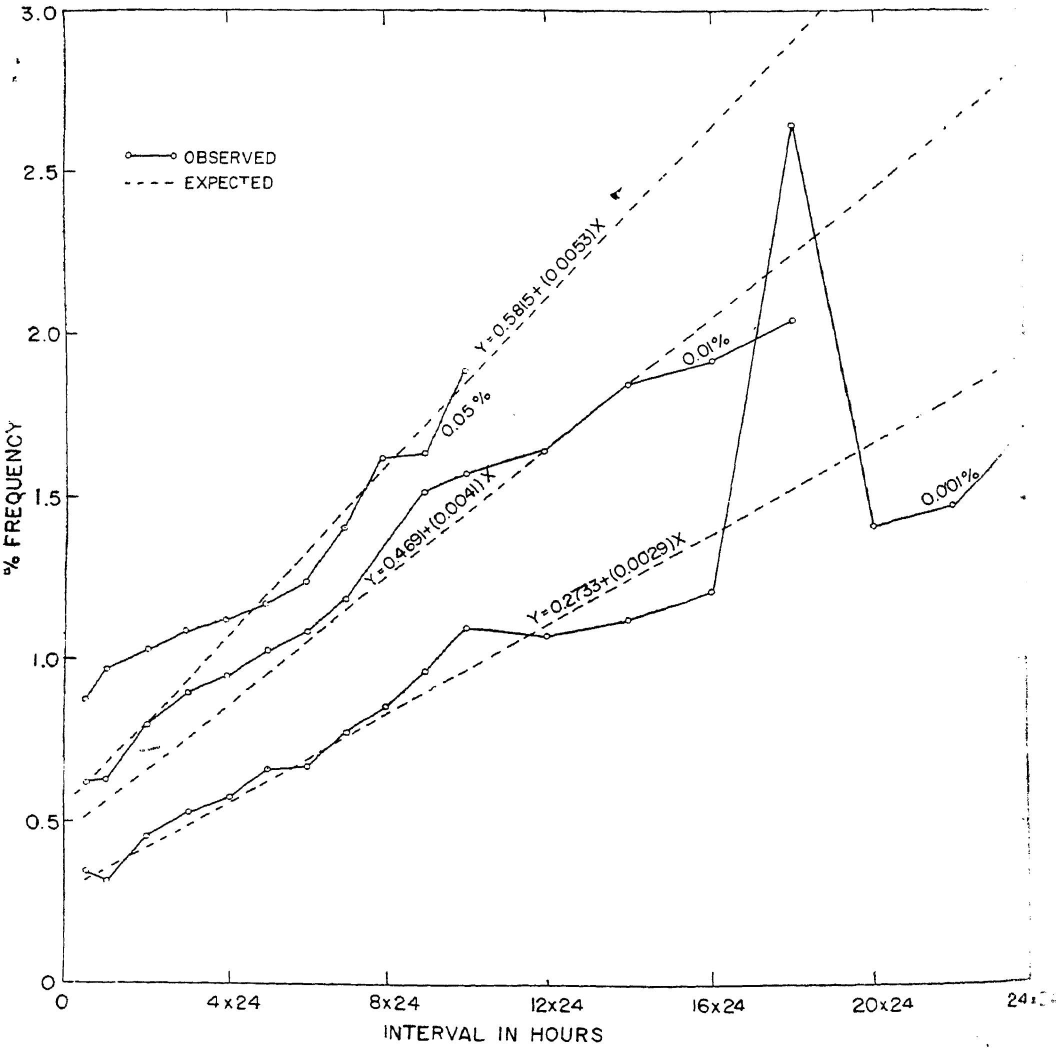
Males of *Poekilocerus pictus* collected from the environs of Manasa Gangotri, Mysore (India) formed the material for the present study. The animals were injected with 0·05 ml. of 0·001%, 0·01% and 0·05% aqueous cadmium chloride solution abdominally and the animals survived upto 24 days, 18 days, and 10 days, respectively, for the above three concentrations. Testes Squash chromosomal preparations were made and stained in Heidenhain's Iron Hematoxylin.

The qualitative analysis has revealed the presence of different types of chromosomal aberrations, such as, stickiness and clumping (Fig. 1), pseudobridge formation at anaphase-I (Fig. 2) and at anaphase-II (Fig. 3), tetraploidy at metaphase-II (Fig. 4), lagging of chromosomes at anaphase-I (Fig. 5) and despiralization of metaphase-II chromosomes (Fig. 6).



FIGS. 1–6. Fig. 1. Diakinesis-extreme clumping (arrows). Fig. 2. Anaphase-I-rope-like pseudobridge (arrow). Fig. 3. Anaphase-II-tetraploidy with pseudo bridge (arrow). Fig. 4. Metaphase-II-tetraploidy with stickiness. Fig. 5. Anaphase-I-lagging (arrow). Fig. 6. Metaphase-II-tetraploidy with despiralization (arrow).

Several chromosome aberrations have been observed[2] in the cultured human leukocytes after treatment with Cadmium sulfide at a concentration of $6 \cdot 2 \times 10^{-2}$ μg/ml of culture fluid. Induction of sticky chromosomes by Cadmium chloride has been reported as early as 1950[3]. Stickiness is a pathological manifestation. The biochemical investigations[4,5] on the effects of Cadmium chloride on DNA in rat and mouse testes showed that there was a decrease in the nucleic acid content. It has been suggested[6] that the depolymerization of nucleic acids is responsible for the production of stickiness. The electron microscopic studies[7] on the Ethidium bromide induced stickiness have shown that the chromosome stickiness is cau to the entanglement of the chromatin fibers b unrelated chromosomes. The present obse have shown that the stickiness is more a morph and mechanical manifestation. Formation of p bridges is one of the direct expression of st The present investigations have shown that bridges are formed due to the terminal stickiness regard to the metaphase-II despiralization, it considered opinion of the authors that the c acts on the coiling system of the chromosomes metaphase-II tetraploidy appears to have been due to the failure of cytokinesis. Generally

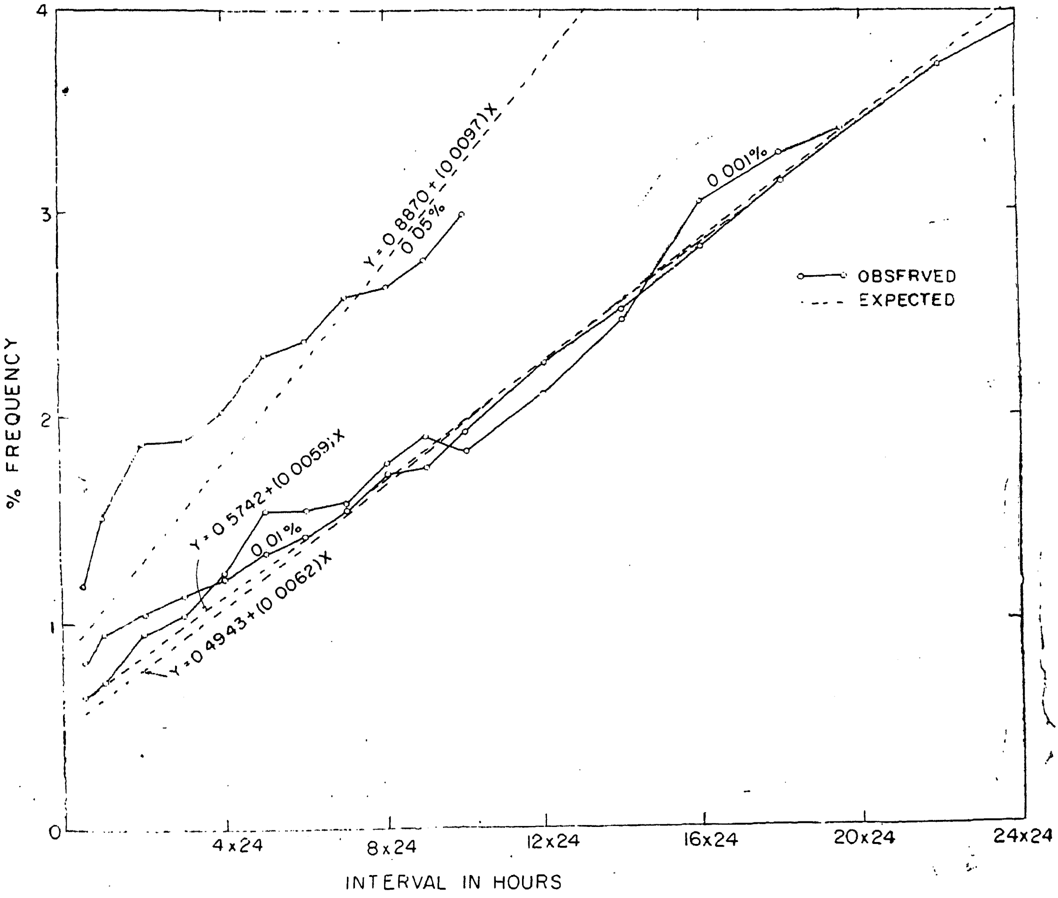


TIME-YIELD RELATIONSHIP OF PSEUDOBRIDGES AT ANAPHASE I STAGE

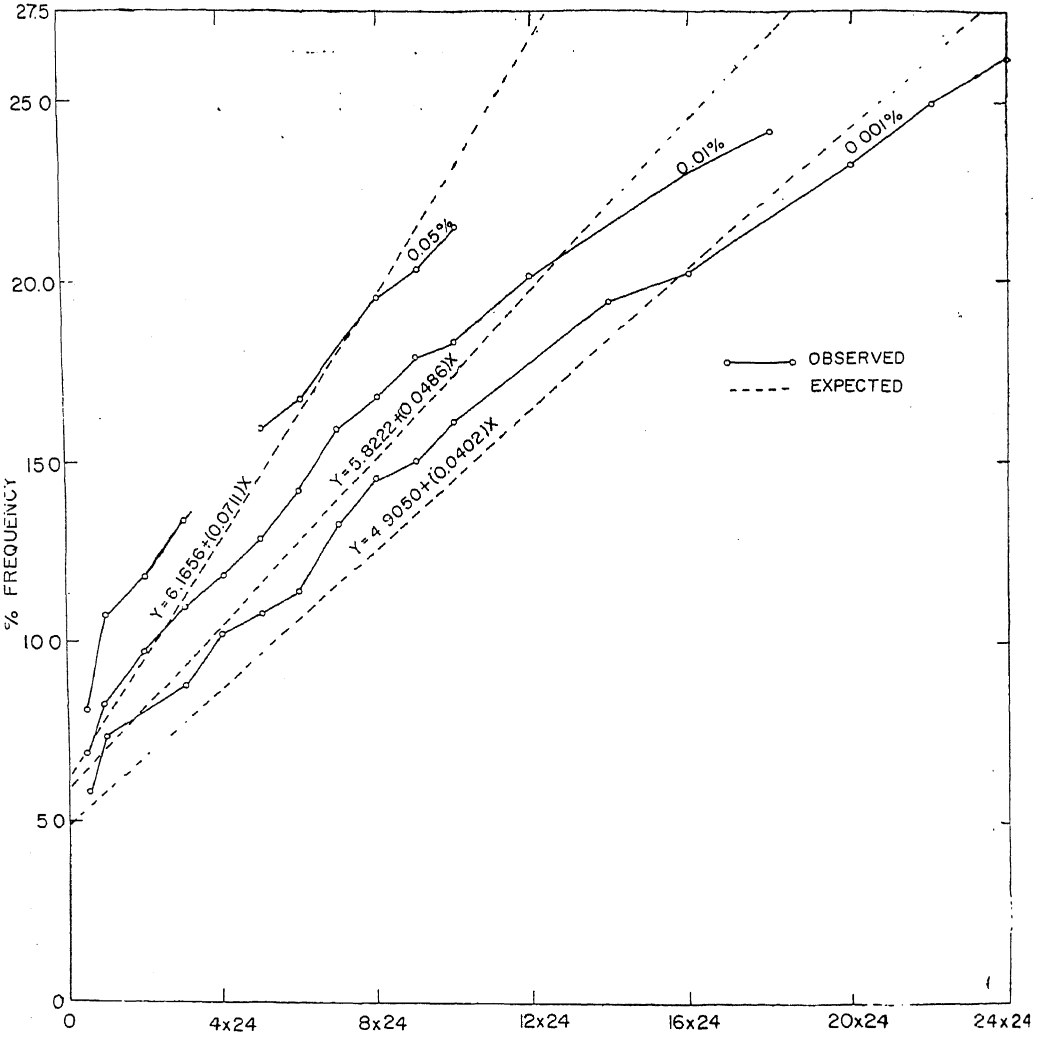
FIG. 7

of chromosomes is encountered in the natural populations of Acridids at a very low frequency. The present observations on the occurrence of laggards to a high degree induced by the pollutant have led the authors to believe that the laggards encountered at anaphase-I are the outcome of the delayed terminalization of interstitial chiasma but not either due to the defective centromeric activity or malfunctioning of the spindle fibers, because, it is proved by the fact that the laggards have not been observed in the anaphase-II stages.

The quantitative analysis of the different chromosomal aberrations induced by cadmium chloride with regard to the time-yield and dose-yield relationships of the anomalies have been studied. The data on these lines have been depicted in Figs. 7, 8 and 9. The present observations have shown that there is a direct correlation between the frequencies of pseudobridges at anaphase-I and anaphase-II and the time interval. Maximum number of pseudobridges have been obtained at 24 days, 18 days and 10 days for 0·001%, 0·01% and 0·05% doses, respectively. Only in the case of 0·001% dose the frequency of anaphase-I pseudobridges gradually increases upto 18 days and then declines. In all the doses of treatment, the percentage frequency of anaphase-II pseudobridges is more than the anaphase-I pseudobridges. The percentage frequency of pseudobridges increases with the increase in dose from 0·001% to 0·01% to 0·05%. The metaphase-II cells are the most susceptible ones for the chemical damage. There is a positive correlation of the cells showing the polyploids with respect to the time and dose, that is, there is an increase in the frequency of polyploids with the increase in the duration of post-interval treatment and with the increase in dose.



TIME-YIELD RELATIONSHIP OF PSEUDOBRIDGES AT ANAPHASE II STAGE



TIME-YIELD RELATIONSHIP OF POLYPLOIDY AT METAPHASE II STAGE

FIG. 9

e biometrical analysis with regard to the straight s of the form Y $= ax + b$ have been fitted to the ta on the timeyield relationships by the method of ast squares for pseudobridges at anaphase-I, anaphase-II and the metaphase-II polyploids. The high degree of correlation between the observed and the expected values in all the cases indicates that the factor time is linearly related to the yield of anomalies ($r =$ 0·8 to 0·9). In calculating the dose-yield relationships no straight lines could be fitted because of the large number of variables that are present in the three different doses. The details of these studies are being worked out.

One of us (KRK) acknowledges with gratitude the Council of Scientific and Industrial Research, New Delhi, for the financial assistance.

1. Gunn, S. A. and Gould, T. C., *The Testis*, Academic Press, New York, 1970, p. 411.
2. Shiraishi, Y., Kurahashi, H. and Yoshida, T. W., *Proc. Jap. Acad.*, 1972, **48**, 133.
3. Avanzi, M. G., *Caryologia*, 1950 *b*, **3**, 234.
4. Webb, M., *J. Reprod. Fert.*, 1972, **30**, 83.
5. Gantayat, S. C. and Patnaik, B. K., *Curr. Sci.*, 1975, **44**, 890.
6. Darlington, C. D., *Nature*, 1942, **149**, 66.
7. Mc Gill, M., Pathak, S. and Hsu, T. C., *Chromosoma*, 1974, **47**, 157.

EFFECT OF ULTRAVIOLET RADIATION ON THE RESPONSE OF *PANICUM REPENS* L. TO INOCULATION WITH *PYRICULARIA* SPP.

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ABSTRACT

Ultraviolet irradiation of *P. repens* leaves before inoculation not only increased their susceptibility to a compatible isolate of *Pyricularia* but also altered their typical resistant response to an incompatible isolate. Irradiated leaves aged faster than unirradiated leaves. However, the effect of UV could not be duplicated by ageing unirradiated leaves before inoculation.

INTRODUCTION

THE response of plants to infection by fungi has been reported to be influenced by ultraviolet irradiation[1-4]. Several effects of UV irradiation on leaves are known[5,6]. The object of the present investigation was to find out if irradiation of *P. repens* leaves before inoculation would alter their response to compatible/incompatible isolates of *Pyricularia* and to study the effect of irradiation in terms of UV induced ageing of the leaves.

EXPERIMENTAL AND RESULTS

A set of 16-20 detached *P. repens* leaves for each treatment was floated on distilled water and irradiated at a distance of 25 cm from a 15 W General Electric germicidal lamp for 1–15 min. The leaves were transferred to 40 ppm benzimidazole solution and inoculated with conidia (10×10^4/ml) of an incompatible (P_1 or M_1) or a compatible (PR) isolate of *Pyricularia*. Irradiated uninoculated controls were also maintained which remained free from infection. The leaves were incubated under diffuse light (12 h light/dark cycle) in a growth room for a total period of 7 days. Daily observations were made for symptom development.

Unirradiated leaves inoculated with the incompatible P_1 isolate showed the typical resistant response. However, irradiated leaves inoculated with the same isolate showed chlorotic ltsions without green islands (Fig. 1 *a*). Examination of the lesions for hyphal development showed that the fungus had generalized in cells of the chlorotic areas. Both unirradiated and irradiated leaves inoculated with the compatible PR developed typical susceptible symptoms with green islands but more chlorosis developed around lesions in the latter treatment (Fig. 1 *b*). The incompatible M_1 did not produce symptoms either in the control or irradiated leaves. The type of symptoms produced by the P_1 and PR isolates, the time of their appearance and the frequency of their development in the different treatments are shown in Table I.

It is evident from the results that the incompatible P_1 isolate produced chlorotic lesions on leaves irradiated for 2 minutes and more as against the typical resistant response in unirradiated leaves. It may be further noted that the number of such lesions increased with increasing irradiation time and the lesions appeared earlier on leaves irradiated for 5 minutes and more than on leaves irradiated for a shorter duration.

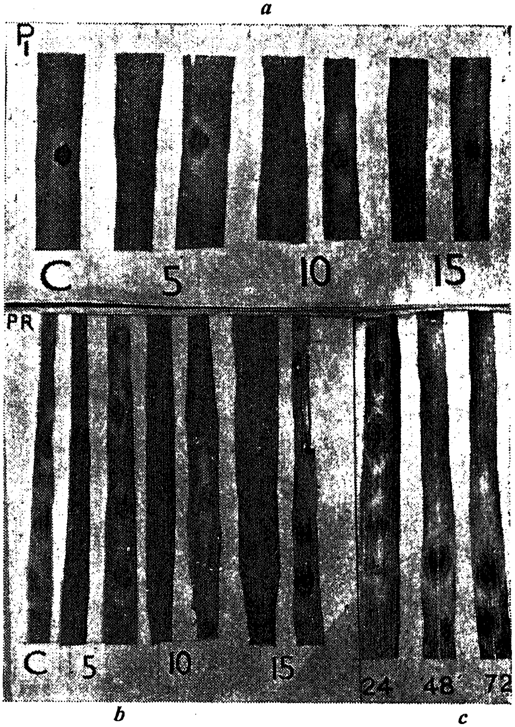

FIG. 1. *a–c.* (*a*, *b*) Symptoms produced on unirradiated (C) and UV irradiated *P. repens* leaves by an incompatible (P_1) and a compatible (PR) isolate of *Pyricularia*; irradiated uninoculated leaves are also shown. (*c*) Symptoms produced by PR on aged leaves. (Refer text for details).

In leaves inoculated with the compatible PR, typical susceptible symptoms developed earlier in

TABLE I

Response of UV irradiated P. repens *leaves to inoculation with an incompatible/a compatible isolate of* Pyricularia

Exposure time (min.)	P_1 (incompatible)		PR (compatible)	
	Day when symptom appeared	Inoculated sites showing symptom (%)	Day when symptom appeared	Inoculated sites showing symptom (%)
0 (control)	3	100·0^{R}	5	50·0^{S}
1	3	100·0^{R}	3	58·5^{S}
2	5	7·5^{C}	3	65·8^{S}
3	5	20·0^{C}	3	82·5^{S}
5	3	30·7^{C}	3	100·0^{S}
10	3	58·5^{C}	3	100·0^{S}
15	3	82·0^{C}	3	100·0^{S}

R — typical resistant response (visible browning). C — chlorotic lesions.
S — typical susceptible response (spindles with green islands).

irradiated than control leaves, and this effect was evident even with one minute irradiation. It may also be seen that the number of lesions increased with increasing irradiation time. In fact, in leaves irradiated for 5 minutes and more, lesions developed at all inoculated sites.

Whether the above noted effects were due to UV induced ageing of the leaves was next studied. Sixteen detached leaves for each treatment were irradiated with UV for 15 minutes and floated on benzimidazole solution as before. One set of unirradiated control and irradiated leaves were left under diffuse light. Seven sets of control and irradiated leaves were left in total darkness. One set of control and irradiated leaves from the dark treatment was removed every 24 h and observed for development of chlorosis as an index of ageing in the leaf tissue. Similar observations were made on leaves kept under light. The response of the leaves to the treatments is shown in Table II. It is obvious from the results that irradiated leaves aged faster than control leaves.

Although irradiated leaves aged faster than control leaves the effect of UV on the response of the leaves to the two isolates could not be duplicated by ageing of unirradiated leaves before inoculation. Unirradiated leaves aged up to 5 days before inoculation did not show any altered response to the incompatible P_1 or the compatible PR. With the latter isolate, however, more chlorosis developed around lesions in leaves aged for 48 h and more (Fig. 1 *c*).

TABLE II

Ageing in UV irradiated P. repens *leaves*

Days after irradiation	Leaves showing chlorosis (%)			
	Light		Darkness	
	Control	Irradiated	Control	Irradiated
1	0	0	0	0
2	0	0	0	0
3	0	0	0	68·7
4	0	0	0	100·0
5	0	0	0	100·0
6	0	0	0	100·0
7	0	0	37·5	100·0
8	0	43·7	..	..
9	0	62·5	..	..
10	0	100·0	..	..

DISCUSSION

Irradiation of *P. repens* leaves with ultraviolet light before inoculation altered their response to inoculation with a compatible/incompatible isolate of *Pyricularia*. The incompatible (P_1) isolate produced chlorotic lesions in irradiated leaves as against the typical resistant response in unirradiated control leaves (Fig. 1 *a*). Irradiation, however, did not alter the response of the leaves to the incompatible M_1 isolate. This isolate appears to have lost the ability to differentiate the infection peg due to mutation[7] and it is not, therefore, surprising that irradiated leaves did not show any altered response to this isolate. Irradiated leaves also showed an increased susceptibility to the compatible PR isolate and a greater number of lesions developed earlier on irradiated than control leaves (Table I).

Results presented in Table II would show that UV irradiated leaves aged faster than unirradiated leaves. However, the altered response of irradiated *P. repens* leaves to the two isolates of *Pyricularia* could not be ascribed to UV induced ageing of the leaves. Ageing of unirradiated leaves before inoculation did not alter their typical response to the compatible PR and the incompatible P_1 isolate[8].

Several suggestions have been made to explain the altered response of UV irradiated leaves to infection by fungi. Buxton *et. al.*[1] speculated that increased infection by *Botrytis fabae* on irradiated broad bean leaves might be due to increased production of foliar exudates that stimulated the pathogen. Others considered that UV irradiation caused injury to the leaf epidermis. It seems unlikely, however, that the above effects of UV could explain the altered response of irradiated *P. repens* leaves to the incompatible P_1 and PR isolates of *Pyricularia*. The effects of UV could not be reproduced with physical injuries to the leaves before inoculation (unpublished observations). In fact, diffusates and extracts of physically injured leaves were less stimulatory to germ tube growth in the compatible PR and more inhibitory to germ tube growth in the incompatible P_1 than those of uninjured leaves[9]. Studying the effects of UV irradiation on resistance of barley (*Hordeum vulgare*) to *Helminthosporium teres* and *H. sativum*, Chakrabarti[10] concluded that increased susceptibility of irradiated leaves was due to partial inactivation of a performed fungal inhibitor present in resistant barley leaves. We have similar evidence to indicate that UV irradiation of *P. repens* leaves before inoculation affects the acccumulation of fungitoxic material in epicuticular waxes. The results of these studies will be published elsewhere.

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1. Buxton, E. W., Last, F. T. and Nour, M. A., *J. Gen. Microbiol.*, 1957, 16, 764.
2. Hey, G. L. and Carter, J. E., *Phytopathology*, 1931, 21, 695.
3. Moseman, J. G. and Greeley, L. W., *Ibid.*, 1966, 56, 1357.
4. Norell, I., *Physiol. Plantarum*, 1954, 7, 797.
5. Dubrov, A. P., *The Genetic and Physiological Effect of the Action of Ultraviolet Radiation on Higher Plants*, Nauka, Moscow, 1968, p. 250.
6. Levitt, J., *Response of Plants to Environmental Stresses*, Academic Press, New York and London, 1972, p. 697.
7. Hilda, A., *Ph.D. Thesis*, Univ. of Madras, 1973.
8. — and Suryanarayanan, S., *Proc. Indian Acad. Sci.*, 1976, 84 B, 215.
9. — and —, *Ibid.*, 1977, 85 B, 257.
10. Chakrabarti, N. K., *Phytopathology*, 1968, 58, 467.

EVOLUTION OF STOMATAL COMPLEX IN EMBRYOBIONTA

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ABSTRACT

A survey has been carried out of the stomatal apparatus in the various 'type' taxa of the Embryobionta as suggested in the classification proposed by Cronquist, Takhtajan, and Zimmermann (1966). The study includes not only the true leaves of higher plants but the scaly leaves of *Psilotum*, *Rhynia*, *Equisetum*, and *Eqhedra* have also been taken into account. It is seen that the stomatal apparatus is considerably different in its organization among the taxa examined suggesting the polyphyletic origin of the diverse groups of the plant kingdom.

INTRODUCTION

A RECORD of the various characters of the leaves, forms the subject-matter of this, an earlier communication (Paliwal *et al.*, 1976) has been widely employed in studying the history of the group. For example, the analysis of the fossil leaf characters has been important in discussions of the origin of the angiosperms (Sinnott and Bailey, 1914; Axelrod, 1952, 1960, 1970; Scott *et al.*, 1960), their subsequent evolution and diversity (Wolfe and Barghoorn, 1960; Takhtajan, 1969; Delevoryas, 1971), and their distribution through time and space (Cain, 1944) and in paleolimatic interpretations (Wolfe and Hopkins, 1967; Axelrod and Bailey, 1969; MaGinitie, 1969; Wolfe, 1971; Dilcher, 1973).

In the present survey, the variation in the organization of the stomatal apparatus has been considered in the Embryobionta as a whole which has been rather ignored (since studies have been mostly restricted to the familial, generic, or specific levels both in the fossil and living forms), except for a brief attempt in this direction by Porsch (1905).

Experimental

Sources of information.—For the study of the stomatal complex in different taxa, materials and illustrations were collected from different books, publications, herbarium sheets, museum and fresh specimens.

Preparation of the material for microscopic study.—The epidermis was peeled away directly from the leaf surface except in such instances where it was difficult to do so. Consequently, such leaves or stems were placed in a slightly modified mixture of Jeffrey (comprising equal volumes of 10% chromic acid and concentrated nitric acid). The cuticular peels thus obtained were thoroughly washed with distilled water and placed in 50% alcohol, stained with Delafield's hematoxylin and passed through a series of alcohol and xylene and finally mounted in piccolyte.

Results

In *Rhynia gwynnee-vaughani*, occurrence of true anomocytic[*] stomata has been reported in a well-documented publication by Pant (1960). In *Marchantia polymorpha* the epidermal cells are isodiametric, pentagonally arranged, with straight cell walls. The stomata are absent and only barrel-shaped pores are present. The epidermal cells in *Anthoceros himalayensis* (sporophytic) are rectangular with undulate cell walls arranged in a linear elongate manner with small and large cells alternating each other. The cells are 0·09 mm long and 0·02 mm broad. The anomocytic, kidney-shaped stomata are raised with length and breadth varying from 0·07–0·06 mm. In *Bryum* sp., *Psilotum nudum, Lycopodium selago, Isoetes* sp., *Ophioglossum reticulatum, Polypodium membranaceum, Marsilea minuta, Salvinia natans, Ginkgo biloba,* and Ephedra *distachya* anomocytic (aperigenous) type of stomata are present. *Equisetum* sp., *Welwitschia bainesii*, Gnetum gnemon, *Bennettites* sp., and *Magnolia grandiflora* possess paracytic stomatal apparatus whereas in the taxa like *Marattia* sp., *Cycas circinalis*, and *Pinus roxburghii* the stomatal complex is of cyclocytic type.

In *Anthoceros* the stomata occur on the sporophytic tissue which is also true for *Bryum* where the stomata are present on the capsule. The stomatal frequency per unit area is maximum (37·5) in *Marsilea minuta* and least (3·57) in *Anthoceros himalayensis* and *Isoetes* sp. (3·84). The stomata are placed at the same level as the epidermis in *Marattia, Polyopodium* and *Gnetum*; are raised in *Anthoceros* and *Magnolia,* and sunken in species of *Isoetes, Equisetum, Cycas, Pinus, Ephedra,* and *Welwitschia*. Conspicuous surface ornamentation is seen on the epidermal and guard cell surfaces in the species of *Equisetum* and *Cycas* as well as in *Ephedra* and *Welwitschia* (*see* Paliwal *et al.,* 1974). In *C. circinalis* a peculiar feature is that the sunken guard cells are present only below the subsidiary cells and the ring of encircling cells lies at about the same level as the subsidiary cells. The monocyclic stomata of *Ginkgo biloba* have their guard cells partially overarched by the inner margins of 4–6 subsidiary cells. Normally, they are further protected by the centripetally directed thickened papillae of the subsidiary cells. In *Ephedra distachya* each stomatal apparatus occurring on the stem have two subsidiary cells which are very distinct with a dense protoplasm and a prominent nucleus. Some of these also have more than two (three or four) subsidiary cells. Thus stomata of two types can be made out: (*a*) those having three or four subsidiary cells surrounding the guard cells and (2) those with only two elongated subsidiary cells; the latter being in majority. At high magnification each stoma reveals its sunken position clearly. The inner concave walls of the guard cells surrounding the pore are thicker than the outer ones. In *Gnetum gnemon* at lower magnification, the stomata were found to be irregularly but closely oriented in different planes. Their number is 20 per sq. mm. as compared to 10 on the stem epidermis of *Ephedra.* Each stoma is surrounded by two subsidiary cells. The two kidney-shaped guard cells are elongated, giving the stomata an oval outline. The stomata appear to be almost at the same level as the epidermal cells themselves. The stomata of *Welwitschia bainesii* are few and scattered irregularly. The guard cells are kidney-shaped and are positioned sunken in relation to the epidermal and the subsidiary cells. The two subsidiary cells are recognizable from the other epidermal cells by their denser cytoplasmic contents.

We failed to get suitable illustrations for the stomatal complex of the fossil taxa like *Hyenia elegans, Sphenophyllum myriophyllum, Protopteridium minutum, Archaeopteris fimbriata, Noegerathia* sp., *Lyginopteris oldhamia, Bennettites* sp. However, from the information we could gather, it became apparent that the guard cells are kidney-shaped in most of them. The inner walls of the guard cells are thick in all the representatives. These are two in number except in *Bryum* sp. where a single oval structure was noticed. The guard cells of *Salvinia* sp. and *Marsilea* sp. are very small as compared to the other taxa studied.

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[*] Terminology after Fryns-Claessens and van Cotthem (1973).

GENERAL COMMENTS AND CONCLUSIONS

Fossil cuticle, as well as cuticle of living leaves, contains a host of characters which may be important in determining relationships at specific, generic and family levels. Workers from all corners have given comprehensive and suggestive accounts on the stomatal types of different monocotyledons, dicotyledons, and gymnosperms (Priestley, 1943; Paliwal, 1965, Stace, 1965; Martin and Juniper, 1970; Sinclair and Sharma, 1971; Dilcher, 1974). Stebbins and Khush (1961) present an account of the stomata of the monocotyledons and considered the stomatal complex systematically in relation to the growth habit, and also the geographic distribution of stomatal types. They suggested that the specialized stomatal complex, once adapted, persisted even though the habitat in which the early evolution of the stomatal complex took place may have changed. Paliwal (1969) and Tomlinson (1974) in their reviews of the usefulness of the stomatal complex development as a taxonomic tool in the monocotyledons disagree with Stebbins and Khush and suggest that it is premature to speculate about the phylogenetic significance of stomatal patterns in the monocotyledons. They further suggest that the stomatal complex has systematic value only when used in combination with several other characters of known taxonomic usefulness.

In this study stomata differ considerably among the taxa studied which suggests the polyphyletic origin of the plant kingdom. For example in *Bryum* sp., *Psilotum nudum, Lycopodium selago, Isoetes* sp., *Ophioglossum reticulatum, Polypodium membranaceum, Marsilea minuta, Salvinia natans, Ginkgo biloba* and *Ephedra distachya* anomocytic (aperigenous) type of stomata are present. *Equisetum* sp., *Welwitschia bainesii, Gnetum gnemon, Bennettites* sp. and *Magnolia grandiflora* possess paracytic stomatal apparatus while in the taxa like *Marattia* sp., *Cycas circinalis,* and *Pinus roxburghii* the stomatal complex is of cyclocytic type. In *Anthoceros,* the stomata occur on the sporophytic tissue which is also true for *Bryum* where the stomata are present on the capsule.

Variation in stomatal frequency (*Anthoceros himalayensis* — 3·57; *Marsilea* — 37·5) and position of guard cells (at the level of epidermis in *Marattia, Polypodium* and *Gnetum*; raised in *Anthoceros* and *Magnolia*; sunken in *Isoetes, Equisetum, Cycas, Pinus, Ephedra* and *Welwitschia* sp.) may all be due to ecological conditions prevailing in the areas where these taxa were growing. Stomata coming in about the epidermal cell level may be due to increasing hydrophytic habits.

Taxa possessing same type of stomata may be due to parallelism or convergence of evolution among them.

ACKNOWLEDGEMENTS

The authors are extremely grateful to colleagues at the laboratory for their kind co-operation and valuable suggestions.

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1. Axelrod, D. I., *Evolution,* 1952, 6, 29.
2. —, In: *Evolution after Darwin,* Vol. I, *The Evolution of Life,* Ed., S. Tax, Univ. of Chicago Press, 1960, p. 227.
3. —, *Bot. Rev.,* 1970, 36, 277.
4. —, and Bailey, H. P., *Palaeogeography, Palaeoclimatol., Palaeoecol.,* 1969, **6**, 163.
5. Cain, S. A., In: *Foundation of Plant Geography,* Harper and Bros., N.Y., 1944, p. 556.
6. Cronquist, A., Takhtajan, A. and Zimmermann, W., *Taxon,* 1966, 15, 129.
7. Delevoryas, T., *Proc. North Amer. Paleontol. Conven.,* Part L, 1971, p. 1660.
8. Dilcher, D. L., In *Vegetation and Vegetational History of Northern Latin America,* Ed. A. Graham, Elsevier Pub. Co., Amsterdam, 1973, p. 39.
9. —, *Bot. Rev.,* 1974, 40, 1.
10. Fryns–Claessens, E. and van Cotthem, W., *Ibid.,* 1973, 39, 71.
11. MacGinitie, H. D., *Univ. Calif. Publ. Geol. Sci.,* 1969, 83, 140.
12. Martin, J. T. and Juniper, B. E., *The Cuticle of Plants,* St. Martin's Press, New York, 1970, p. 347.
13. Paliwal, G. S., "Part I, Structure and Development of Stomata in some Angiosperms, Part II, Stomata in Angiosperms: A Review," *Ph.D. Thesis,* Delhi Univ., 1965.
14. —, *Acta Bot. Neerl.,* 1969, 18, 654.
15. —, "Garg," Meenakshi and Harjal, Nilima, *Curr. Sci.,* 1976, 45, 171.
16. —, Rampal, G. C. and Paliwal, Nilima, *Acta Bot. Indica,* 1974, 2, 37.
17. Pant, D. D., *Proc. of the Summer School of Botany, Darjeeling,* 1960, p. 276.
18. Porsch, O., *Jena,* 1905, p. 196.
19. Priestley, J. H., *Bot. Rev.,* 1943, 9, 393.
20. Scott, R., Barghoorn. E. S. and Leopold, E. B., *Am. J. Sci.,* Bradley Volumes, 1960, **258 A**, 284.
21. Sinclair. C. B. and Sharma, G. K., *Jour. Tenn. Acad. Sci.,* 1971. 46, 2.
22. Sinnott, E. W. and Bailey, I. W., *Am. J. Bot.,* 1914. 1, 441.
23. Stace, C. A., *Bull. Brit. Mus. Bot.,* 1965, **4**, 1.

24. Stebbins, G. L. and Khush, G. S., *Am. J. Bot.*, 1961, 48, 51.
25. Takhtajan, A., *Flowering Plants Origin and Dispersal,* 1969, p. 310.
26. Tomlinson, P. B., *Taxon,* 1974, 23, 109.
27. Wolfe, J. A., *Palaeongeography, Paleoclimatol, Palaeoecol.,* 1971, 9, 27.
28. Wolfe, J. A. and Barghoorn, E. S., *Am. Jour. Sci.*, Bradley Volume, 1960, 258 A, 388.
29. — and Hopkins, D. N., In : *Tertiary Correlations and Climatic Changes in the Pacific,* Symp. Pacific Sci. Congr., 11th, Tokyo, Aug.–Sept., Ed. K. Hatai, 1967, 25, 67.

RECENT RESEARCHES IN PLANT SCIENCES

S. S. BIR

Convenor of the Symposium

Department of Botany, Punjabi University, Patiala 147 002

A NATIONAL symposium on "Recent Researches in Plant Sciences", was held at the Department of Botany, Punjabi University, Patiala, during January 20–22, 1977. Ninety plant scientists representing 30 universities and institutes from all over India took part in the symposium, inaugurated by Mrs. I. K. Sandhu, Vice-Chancellor, Punjabi University. Apart from presentation of papers, special and plenary lectures by eminent botanists constituted the important part of the symposium.

Prof. P. N. Mehra discussed the structural and morphological variability in the Himalayan orchids. Role of the structure and behaviour of chromosomes in relation to the amount of DNA and other constituents in a species was explained by Prof. A. K. Sharma. He formulated a new concept of chromosomal dynamism in evolution and stressed that the flexibility of chromosome behaviour has high potentialities in genetic engineering for crop improvement. Dr. T. N. Khoshoo stressed the need for the improvement of ornamentals in India for earning foreign exchange. Genetical basis of monoecism and dioecism in plants was discussed by Prof. R. P. Roy and he made a strong case for exemplifying indigenous material in teaching of cytogenetics. Prof. V. Puri pleaded for the avoidance of the present practice of forcing fossil specimens into descriptions of modern genera. Prof. T. V. Desikachary pointed out that in spite of fine structure studies in algae, we are still in the beginning of our search for the possible ancestors of land plants from aquatic plants and heterotrichous condition is an important step in this direction. Production of adventitious roots, according to Prof. K. K. Nanda, is controlled by a balance between auxin, IAA oxidase, inhibitors of IAA-oxidase and bound auxins in the system. Prof. C. P. Malik explained the metabolic changes that take place during pollen germination. An elaborate account of the recent palaeobotanical discoveries and their phylogenetic significance was discussed by Prof. D. D. Pant whereas the role of enzymes in *Zizyphus* gall tissues was outlined by Prof. H. C. Arya. A strong plea for adopting an integrated approach in taxonomy by utilizing data from other aspects of plant studies was made by Prof. B. M. Johri.

Nearly 100 research communications dealing with morphology, cytogenetics, biosystematics, anatomy, taxonomy plant functions and diseases covering both basic and applied aspect were presented and discussed during 10 academic sessions of the 3-day symposium.

As a result of panel discussions on the concluding day, the following recommendations with regard to teaching and research in plant sciences have been made :

1. Basic researches should be strengthed in the universities and there should be no strings attached to research.
2. While planning and conducting basic researches, plants of economic importance should be selected. Further there should be an inter-disciplinary approach for finding solutions to problems of plant productivity. This could best be achieved through teacher and scientist exchange programme.
3. Researches on lower groups of plants with particular reference to teridophytes need to be promoted.
4. Local and regional floras of the country must be compiled, and work on plant taxonomy, systematics and phytogeography should be promoted. by U.G.C.
5. Work on preparation of 'Chromosome Atlas' of plants of various regions of the country is urgently needed.
6. Researches on forest biology, environmental botany, pollen physiology, seed treatment, plant nematology and reproductive biology need to be strengthened.

7. There should be stress on fundamental and evolutionary approach in teaching of Botany.
8. Cytogenetics, plant geography, ecology and taxonomy need to be given more weightage at undergraduate level whereas forest biology, economic botany, palaeobotany, mathematics, biostatics, biochemistry and biophysics should form important part of the postgraduate curriculum.
9. Proper practical manuals with examples from local flora must be compiled for different levels of botany teaching.
10. With the possible introduction of 10 + 2 + 3 system the syllabi at school and undergraduate levels need to be suitably upgraded. Integrated syllabi with interdisciplinary bias must be evolved from plus two stage to M.Phil.

Since the flora of the country is undergoing rapid change due to the highly accelerated biotic factors, there is an immediate need to set up regional plant conservatories for preserving the wild germ plasm ta be used in future for plant improvement. State and district level museums and botanic gardens must be established for creating interest of general public in plants for habitat improvement and economic progress. Facilities available in nearby institutions must be made aceessible to all the workers. Finally, the research centres must pay attention to the local problems.

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LETTERS TO THE EDITOR

ROTATIONAL ANALYSIS OF THE $A^3\Pi_0{}^+ \rightarrow X^1\Sigma^+$ SYSTEM OF InI MOLECULE

THE spectrum of Indium monoiodide in the region λλ 3948 - 4293 Å was studied at low dispersion by Wehrli[1] (1934) and Wehrli and E. Miescher[2] (1934). Barrett and Mandel[3] (1958) studied the spectrum of InI molecule in the microwave region in absorption and obtained the rotational constants for the ground state of the molecule. The present work was undertaken to study the exact nature of the excited state involved in the emission of A → X band system of the molecule and to determine the rotational constants of the excited state. Rotational analysis of (0, 0), (0, 1) and (1, 0) bands of the A → X system has been carried out and results obtained are reported here.

The spectrum of Indium monoiodide was excited in a high frequency discharge by keeping pure Indium metal in the presence of iodine vapours in a conventional type of a quartz discharge tube. The spectrum was photographed on Ilford N-40 plates in the seventh order of a two meter plane grating spectrograph (Carl-Zeiss) at a resolution of about 3 × 10^5 and a reciprocal dispersion of 0·35 Å/mm. Exposure time of about five hours for a slit-width of 15 microns was adequate to record the spectra of sufficient intensity. Measurements were made on Abbe Comparator using iron-arc standards.

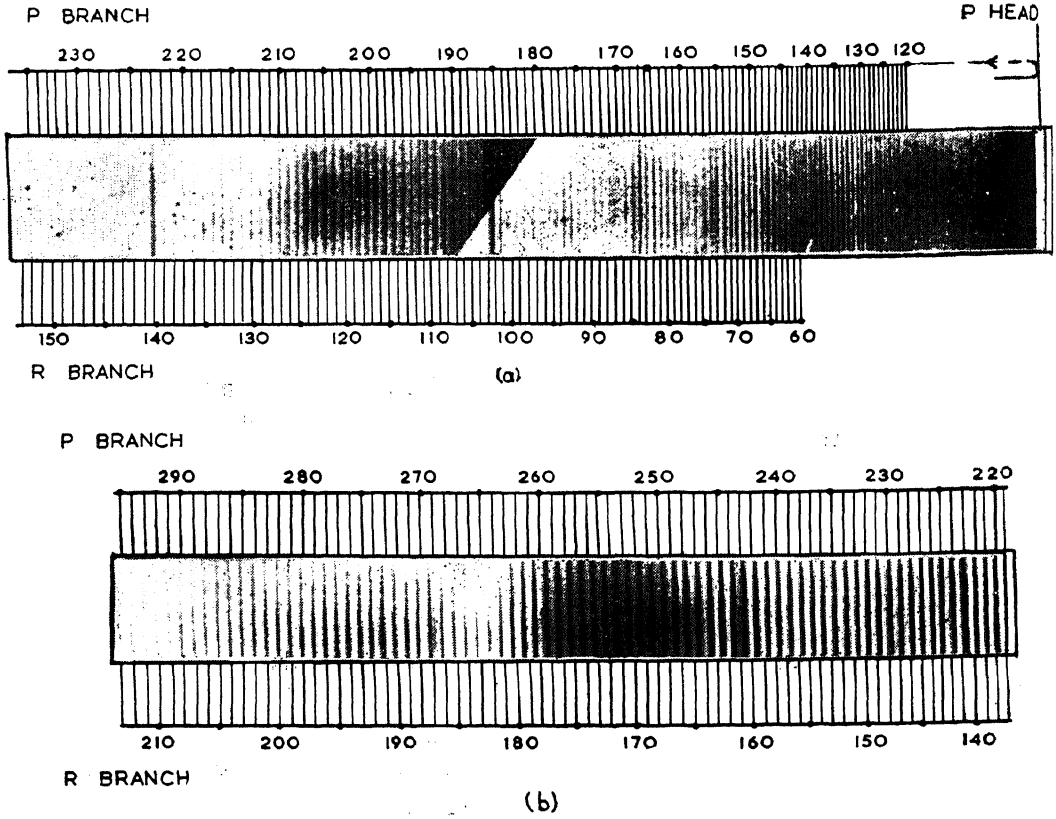

FIG. 1.

FIG. 1 (*a*) and (*b*). Rotational structure of (0, 0) band A → X system of InI molecule taken at a dispersion of 0·35 Å/mm.

In the A – X system of InI molecule, (0, 0), (0, 1) and (1, 0) bands degraded to violet were analysed. These bands reveal the presence of single P and R branches of which P is the head forming branch (Fig. 1). The rotational analysis has been carried out by standard methods (Herzberg[4], 1955 and Younger and Winans[5], 1960). Analysis of (0, 1) band at 4072·7 Å was carried out by comparing the combination relations for the common upper and lower state respectively of (0, 0) band at 4098·5 Å. The rotational constants for the three bands of A → X system obtained in the present work are given below:

Band	ν cm^{-1}	B′ cm^{-1}	B″ cm^{-1}	D′ cm^{-1}	D″ cm^{-1}
(1, 0)	24548·535	$0\cdot0374_2$	$0\cdot0368_1$	$0\cdot12 \times 10^{-7}$	$0\cdot087 \times 10^{-7}$
(0, 0)	24393·853	$0\cdot0377_2$	$0\cdot0368_2$	$0\cdot087 \times 10^{-7}$	$0\cdot075 \times 10^{-7}$
(0, 1)	24216·705	$0\cdot0376_1$	$0\cdot0364_1$	$0\cdot1 \times 10^{-7}$	$0\cdot06 \times 10^{-7}$

Molecular constants of InI molecule obtained from the present analysis are given below along with the microwave data.

State	B_e cm^{-1}	r_e Å	$\triangle G_{\frac{1}{2}}$ cm^{-1}	α_e cm^{-1}
$A^3\Pi_0$	$0\cdot0376_2$	$2\cdot710_2$	$155\cdot7_2$	$0\cdot6 \times 10^{-4}$
$X^1\Sigma^+$	$0\cdot0362_2$	$2\cdot770_1$	$176\cdot1_2$	$1\cdot3 \times 10^{-4}$
	*0·0368	*2·754	—	$1\cdot04 \times 10^{-4}$

The ground state configuration of Indium monoiodide molecule can be written as $z\sigma^2\ y\sigma^2\ w\pi^4\ x\sigma^2$ analogous to those of halides of the same group (InCl, InBr, InF) giving rise to $^1\Sigma^+$ ground state. The excited electron configuration is $z\sigma^2 y\sigma^2 w\pi^4 x\sigma v\pi$ which gives rise to $^1\Pi$ or $^3\Pi$ state. $^1\Pi - {}^1\Sigma^+$ transition is attributed to C → X system which is analogous to those of similar molecules. However $^1\Pi$ state is repulsive in the case of InI molecule which gives a continuum at 3180 Å. The $^3\Pi$ state belongs to Hund's case (*a*) due to its large coupling constant (648·9 cm^{-1}). Hence $^3\Pi_0$, $^3\Pi_1$ and $^3\Pi_3$ states are analogous to $^1\Sigma$, $^1\Pi$ and $^1\triangle$ states respectively. $^3\Pi_2 \rightarrow X^1\Sigma^+$ transition is a forbidden one whereas $^3\Pi_1 \rightarrow {}^1\Sigma^+$ has been ascribed to the B → X system. Hence $^3\Pi_0 \rightarrow {}^1\Sigma^+$ transition may be attributed to the A – X syxtem of InI molecule. The appearance of single P and R branches in case of (0, 0), (0, 1) and (1, 0) bands confirms this assignment.

The authors are thankful to Prof. M. M. Patel for his keen interest and useful discussions during the course of this work.

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Baroda,
February 7, 1977.

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*Microwave data (1958).

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1. Wehrli, M., *Helv. Phy. Acta*, 1934, **7**, 611.
2. – and Miescher, E., *Ibid.*, 1934, **7**, 298.
3. Barrett, A. H. and Mandel, M., *Phy. Rev.*, 1958, **109**, 1572.
4. Herzberg, G., *Molecular Spectra and Molecular Structure* (*Spectra of Diatomic Molecules*), Published by D. Van Nostrand Co., N.Y., 1955.
5. Youngner, P. and Winans, J. G., *Journal of Mol. Spectroscopy*, 1960, **4**, 23.

COPPER COMPLEXES OF PHTHALHYDRAZIDE-5-AZO-2-NAPHTHOL

Our previous work on metal chelates of *o*-hydroxyazo compounds[1] led us to a tridentate ligand, phthalhydrazide-5-azo-2-naphthol, which unlike the bidentate *o*-hydroxyazo compounds, forms stable metal chelates with a variety of metal ions. Complexes of this tridentate ligand with Copper (II), wherein the fourth co-ordination position of the metal ion is occupied by ammonia or a heterocyclic base (pyridine or *α*, *β* or *γ*-picoline) have been characterised.

Experimental

Phthalhydrazide-5-azo-2-naphthol was prepared as follows: 3-Nitrophthalic anhydride was treated with hydrazine sulphate in presence of sodium acetate. The resulting 5-nitrophthalhydrazide was reduced with ammonium sulphide to yield pale yellow 5-aminophthalhydrazide (luminol) in 90% yield[2]. This was diazotised and coupled with *β*-naphthol when phthalhydrazide-5-azo-2-naphthol[3] resulted in 70% yield. Recrystallised from ethyl alcohol m.p. 305–307° C.

The copper complex containing ammonia was prepared as follows: Copper(II) chloride dihydrate (0·85 g; 0·005 mole), dissolved in the minimum amount of water, was added with stirring to a solution of the ligand (1·65 g; 0·005 mole) in liquor ammonia (50 ml). The mixture was stirred for half an hour, then cooled, filtered, and the crystalline residue washed with water. Yield: 1·6 g. Complexes containing the heterocyclic bases were prepared similarly, by using 150 ml. of the base in place of liquor ammonia. The mixed solutions in these cases were also warmed to about 50°, before cooling. Yields are noted in Table I. Since the complexes are only sparingly soluble in common organic solvents, they could not be recrystallised, but were washed with hot alcohol, before sucking dry. The compounds did not melt or decompose until 330°.

Carbon and hydrogen contents of the ligand and the complexes were determined microanalytically, and metal content of the complexes by usual gravimetric procedure, after decomposing them with concentrated sulphuric-nitric acid mixture. A list of complexes prepared and their analytical data are given in Table I.

TABLE I

Sl. No.	Compounds	Yield %	Colour	Analytical data* C	H	Cu
1.	Phthalhydrazide-5-azo-2-naphthol (H_2L)	65	deep red	66·65 (67·05)	3·18 (3·32)	
2.	[CuL (NH_3)]	80	brownish violet	51·89 (52·62)	3·62 (3·65)	15·20 (15·4
3.	[CuL (pyridine)]	75	black	59·18 (58·41)	3·75 (3·60)	12·98 (13·4
4.	[CuL (α-picoline)]	70	brown	58·28 (59·21)	3·71 (3·90)	12·58 (13·0
5.	[CuL (β-picoline)]	70	brown	57·97	3·65	12·30
6.	[CuL (γ-picoline)]	70	brown	57·26	3·84	12·71

+ Calculated values are in parentheses.

Thermograms were obtained on a Stanton recording thermobalance. A pye-Unicam U.V.-Visible spectrophotometer was used for recording U.V. spectra in DMF. Infrared spectra of KBr pellets were recorded using a Perkin Elmer Model 337 spectrophotometer.

Results and Discussion

The complexes prepared are non-conducting in DMF, and do not contain the counter anion of the copper salt used for synthesising the complexes. On the basis of their analytical data these complexes are represented in Table I. The complexes have been further characterised on the basis of infrared spectral evidence.

Investigations in the past have revealed that *o*-hydroxyazo compounds (for example phenylazo-β-naphthol) exist predominantly in their quinone-hydrazone form, which is intramolecularly hydrogen bonded[4,5]. Due to this strong hydrogen bonding the quinone carbonyl stretching frequency remains considerably low, and is at about 1615 cm^{-1} variety of *o*-hydroxyazo compounds. There also strong evidences to show that phthalhyd exists almost entirely in the monohydroxy fo On the basis of such evidences the ligand, ph hydrazide-5-azo-2-naphthol can be represent structures A and B in Fig. 1. In view of tautomerism shown, the amide carbonyl stret frequency would also be expected to be low. conformity with the above two structures, ligand shows two strong absorptions in the d bond region at 1630 and 1615 cm^{-1}, assignab the amide carbonyl and quinone carbonyl re tively of the ligand.

As should be expected from the hydrogen b structure, a strong and considerably broad extending from 3500 to 2400 cm^{-1} (maximu 3000 cm^{-1}) is observed in the spectrum of ligand. Although O–H, N–H and the molecularly hydrogen bonded N–H–O group could be contributing to this band, only the mentioned can be responsible for the obser extension of the band to such low wave numbe

In the spectra of the metal complexes, the carbonyl bands of the ligand, are shifted to ab 1660 and 1575 cm^{-1}. Metal ligand bonding, a structure C in Fig. 1, can adequately explain shift in the carbonyl stretching frequencies. T quinone carbonyl of the ligand, perturbed thro co-ordination to the metal, shows a depre carbonyl stretching frequency at about 1575 c On the other hand metal chelation fixes tautomeric form A of the ligand, thus strengthen its amide carbonyl (by obviating tautome involving structure B) and consequently raising stretching frequency to about 1660 cm^{-1}. Spec in the 3500–2400 cm^{-1} region also support structure, since in the place of the very br band of the ligand, a considerably narrower ba (3500–2900 cm^{-1}) with maximum at about 3300 c occurs in the spectra of the complexes. Structure would demand such a change, since the chelated hydrogen of the ligand has been replaced by the metal atom (together with the O–H hydrogen) and hence the band is now caused only by the N–H group which, from the breadth of the band, appears to be involved in weak intermolecular hydrogen bonding.

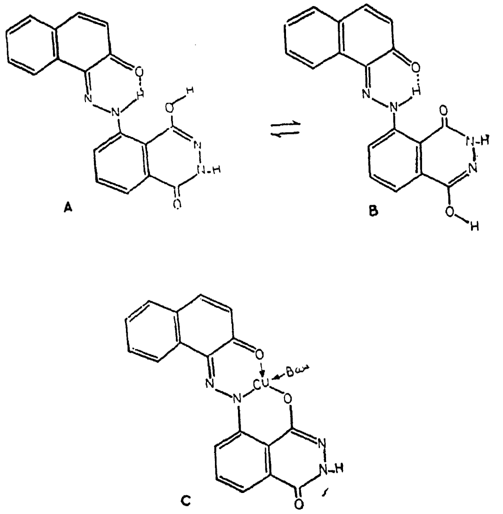

FIG. 1

The complex, [CuL(NH$_3$)], when boiled with caustic alkali liberated the expected amount of ammonia. Thermograms of the complexes [CuLB] (B = base) reveal that the heterocyclic base is expelled preferentially and quickly in a single step at about 340° C, after which the rest of the molecule decomposes slowly.

The U.V. spectrum of the complex [CuL(NH$_3$)] is very similar to that of the ligand. Hence it is evident that no rearrangement of the ligand occurs during chelation.

A copper chelate of the N-methyl derivative of the title ligand has been previously reported[9]. Although no structural study was attempted, the chelate has been assumed to have an analogous structure shown in Fig. 1 C.

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1. Thankarajan, N. and Krishnan Kutty, K., *Ind. J. Chem.* (In press).
2. Huntress, E. H., Stanley, L. N. and Parker, A. S., *J. Am. Chem. Soc.*, 1934, **58**, 241.
3. Dziomko, V. M., Markovich, I. S. and Zelichenok, S. L., *C.A.*, 1968, **69**, 48967 x.
4. Wiley, R. H. and Jarboe, (Jr.) C. H., *J. Am. Chem. Soc.*, 1955, **77**, 403.
5. Venkataraman, K., *The Chemistry of Synthetic Dyes*, Academic Press, 1970, **3**, 253.
6. Elvidge, J. A. L. and Redmcu, A. P., *J. Chem. Soc.*, 1960, p. 1710.
7. Sheinker, Yu, N., Gortinskaya, T. V. and Sycheva, T. P., *Zhur. Fiz. Khim.*, 1957, **31**, 599.
8. Bellamy, L. J., *The Infrared Spectra of Complex Molecules*, Methuen and Co., London, 1954, p. 105.
9. Drew, H. D. K. and Garwood, R. F., *J. Chem. Soc.*, 1937, p. 1841.

Ni(II), Cu(II) AND Pd(II)-CHELATES OF N-HYDROXYETHYLPYRROLIDENEIMINE SCHIFF BASE

N-HYDROXYETHYLPYRROLIDENEIMINE (abbr. H_2P) acts as a bidentate Schiff base and forms solid chelates with Ni(II), Cu(II) and Pd(II). The chelates have been characterised by elemental analysis, magnetic susceptibility measurements, electronic and infrared spectra.

H_2P was synthesised by refluxing an equimolecular mixture of ethanolamine and pyrrole-2-carboxaldehyde in benzene for an hour at 110° C. Water was removed azeotropically with benzene. After distilling out the benzene a yellow solid m.p. 72° was obtained. It gave satisfactory C, H and N analysis.

The metal chelates were prepared by refluxing a mixture of hydrated metal (II)-acetates and H_2P in 95% ethanol for two hours. The solid material formed was filtered, washed with ethanol, dried and recrystallised from methanol. The metal chelates gave satisfactory elemental analysis and display 1 : 2 metal-ligand stoichiometry.

Cu(II)-chelate was found paramagnetic (μ_{eff} 1·87 B.M. at 303° K) while Ni(II)– and Pd(II)-chelates diamagnetic.

The electronic absorption spectra of Ni(II)-chelate in methanol exhibit two bands at around 16200 and 17500 cm^{-1} suggesting the square-planar configuration of the Ni(II)-chelate[1]. The Cu(II)-chelate solution in methanol consist of two broad bands at 16700 and 24200 cm^{-1} which indicate the square-planar geometry of its molecule[2]. Pd(II)-chelate greatly favours a square-planar configuration[2]. Solution spectra of Pd(II)-chelate indicate three bands at 22200, 26400 and 30500 cm^{-1} assignable to the transitions $^1A_{1g} \rightarrow {}^1B_{1g}$; $^1A_{1g} \rightarrow {}^1E_{1g}$ and $^1A_{1g} \rightarrow {}^1A_{2u}$, respectively which reveal a square-planar configuration of the compound. Thus H_2P functions as a bidentate ligand in all the three metal chelates of interest.

In the i.r. spectra of H_2P, three bands were observed at 3610, 3310 and 1600 cm^{-1} assignable to νOH, νNH and νC=N respectively. νC=N of H_2P around 1600 cm^{-1} was shifted to lower frequency side (1590 cm^{-1})[4] showing the participation of the azomethine nitrogen in complexation. The appearance of a new broad band at 600 cm^{-1} suggest M–N bonding in the chelates. Thus the metal-chelates may be represented by the structure shown in Fig. 1.

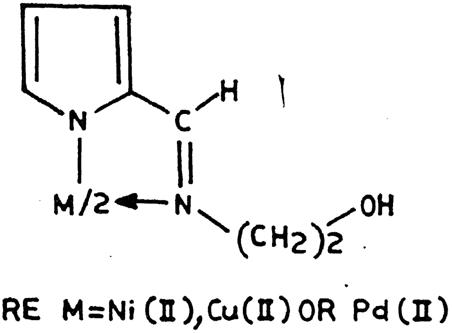

FIG. 1. Metal chelates of N-hydroxyethyl pyrrolideneimine Schiff base.

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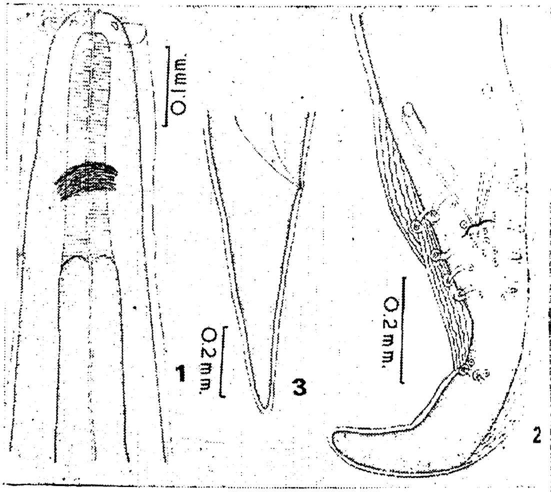
1. Cotton, F. A. and Wilkinson, G., *Advanced Inorganic Chemistry*, 1966, p. 881.
2. Sacconi, L., *J. Inorg. Nucl. Chem.*, 1961, 19, 73.
3. Yamada, S., *Coordin, Chem. Review*, 1966, 1, 415.
4. Daasch, L. W. and Hanninen, U. E., *J. Am. Chem. Soc.*, 1950, 72, 3673.

ON A NEW AVIAN NEMATODES, *PHYSALOPTERA ACCIPITERI* SP. NOV. (FAMILY : PHYSALOPTERIDAE LEIPER, 1908) FROM A SHIKRA, *ACCIPITER BADIUS* (GMELIN) FROM LUCKNOW

A FEW specimens were collected from the gizzard of the bird, *Accipiter badius*. These specimens represent a new species and are designated *Physaloptera accipiteri* sp. nov. The nematodes were fixed in alcohol-formaline, acetic acid and cleared in lacto-phenol.

Physaloptera accipiteri sp. nov. (Figs. 1–3).

Description.—Body fairly stout (Fig. 1). Cuticle of anterior region loosely attached and reversible prepuse-like. Mouth with two papillae on external surface and two teeth on inner surface. Head without ornamentation. Oesophagus consisting of two parts, an anterior shorter, narrow muscular portion and a posterior longer, wider glandular portion. Cuticle thick, transversely striated.



FIGS. 1–3. *Physaloptera accipiteri* sp. nov. Fig. 1. Anterior end of male. Lateral view: Fig. 2. Posterior end of male. Ventral view. Fig. 3. Female tail. Lateral view.

Male.—Caudal alae well developed extending upto tip of tail (Fig. 2). Tail conical. Fifteen pairs of caudal papillae with four pairs preanal and eleven pairs postanal. Of preanal papillae, two pairs pedunculated and two pairs sessile. Of postanal papillar, four pairs pedunculated and seven pairs sessile. Of pedunculated postanal papillae, three pairs lying near anus and one pair lie midway between close and posterior end. Spicule short, tubular, similar and equal. Gubernaculum absent.

Female.—Tail conical (Fig. 3). Vulva pre-equatorial, Uterus filled with numerous thin-shelled eggs.

Discussion.—The new form is referred to the genus *Physaloptera* Rudolphi, 1918. The following species are known from avian hosts from India, *viz.*, *Physaloptera alata Rudolphi*, 1819 ; *P. acuticauda* Molin, 1860 ; *P. crosi* Seurat, 1914 ; *P. galinieri* Seurat, 1914 ; *P. rapacis* Monning, 1921 ; *P. reevesi* Chu, 1931 ; *P. losseni* Ortlepp, 1937 ; *P. mexicana* (Caballero, 1937) Lucker, 1956 ; *P. brachycerca* Kreis, 1938 ; *P. buckleyi* Ali, 1961 ; *P. microcauda* Ali, 1961 ; *P. indiana* Ali, 1961 and *P. sultanae* Ali, 1961.

The new form differs from all these known species of the genus *Physaloptera* except *P. crosi* in having equal spicules instead of subequal or unequal. The new form differs from *P. crosi* in the number and arrangement of caudal papillae and in the relative size of various organs. Accordingly it is regarded as a new species with the specific name, *Physaloptera accipiteri* sp. nov.

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1. Ali, S. M., *Jour. Helminth.*, 1961, 35, 49.
2. Chu, T. C., *Jour. Parasit.*, 1931, 17, 155.
3. Cram, E. B., *Bull. U.S. Nat. Mus.*, 1927, 140, 1.
4. Kreis, H. A., *Zentr. Bakt. I. Abt. Orig.*, 1938, 142, 90.
5. Lucker, J. T., *Proc. Helminth. Soc. Wash.*, 1956, 23, 1.
6. Ortlepp, R. J., *Jour. Vet. Sci. and Ani. Ind.*, 1937, 9, 71.
7. Yamaguti, S., *Systema Helminthum*, Parts I and II, 1961, 3, 1.

ON A NEW AVIAN NEMATODE, *PORROCAECUM (P.) ACCIPITERI* SP. NOV. (FAMILY: HETEROCHEILIDAE RAILLIET ET HENRY, 1915) FROM A SHIKRA, *ACCIPITER BADIUS* (GMELIN) FROM LUCKNOW

SPECIMENS were collected from the intestine of a bird, *Accipiter badius* (Gmelin). These specimens represent a new species and are designated, *Porrocaecum* (*P.*) *accipiteri* sp. nov. The nematodes were fixed in alcohol-formaline, acetic acid and cleared in lactophenol. For the end-on view, the worms were cut with a sharp blade and rolled into the desired position under the cover glass.

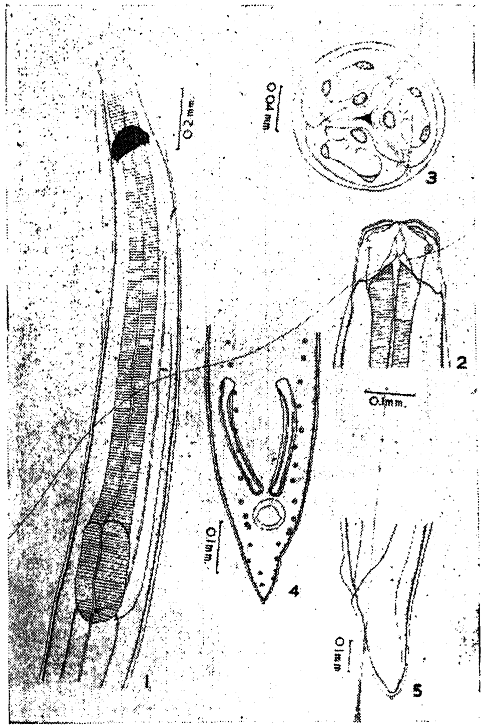
Porrocaecum (*P.*) *accipiteri* sp. nov. (Figs. 1–5).

Description.—Body large, stout and elongated (Fig. 1). In end-on view, (Fig. 3) mouth surrounded by three lips and two sub-ventral each bearing two papillae. Pulp of dorsal lip with two flattened expanded process, Interlabia triangular with broad bases. Dentrigerous ridges extremely fine. Lateral alae present throughout body. Oesophagus more or less uniformly thick with a short ventriculus. Intestinal caecum lying along side of oesophagus anteriorly. Oesophageal appendix absent. Cuticle transversely striated.

Male : Tail conical (Fig. 4). Twenty-nine pairs of sessile caudal papillae with twenty-three pairs preanal, one pair adanal and five pairs postanal. First postanal papillae double, lying immediately behind cloaca, other near tip of tail arranged in tandem by pairs. Spicules equal, similar and alate. Gubernaculum absent.

Female : Tail conical (Fig. 5). Vulva pre-equatorial.

Discussion : Skrjabin, Shikobalova and Mozyovoi (1951) divided the genus *Porrocaecum* Railliet et Henry, 1912 into two subgenera *Porrocaecum* and *Laymanicaecum*. The subgenus *Porroceacum* is characterised by the absence of a gubernaculum, the subgenus *Laymanicaecum* by the presence of one. Due to the absence of a gubernaculum the new form comes under the subgenus *Porrocaecum*.



FIGS. 1–5. *Porrocaecum* (*P.*) *accipiteri* sp. nov. Fig. 1. Anterior end of male. Lateral view; Fig. 2. Anterior end of male. (Magnified) Lateral view; Fig. 3. End-on view; Fig. 4. Posterior end of male. Ventral view; Fig. 5. Female tail. Lateral view.

Seven species *viz.*, *Porrocaecum* (*P.*) *crassum* (Deslong-champs, 1824); *P.* (*P.*) *depressum* (Zedar, 1880); *P.* (*P.*) *ardeae* (Froelich, 1802) Baylis, 1936; *P.* (*P.*) *wui* (Hsü, 1933); *P.* (*P.*) *cheni* (Hsü, 1933); *P.* (*P.*) *haliasturi* Gupta, 1960 and *P.* (*P.*) *indica* Subramanian, 1969 have been described from Asiatic birds. The new form differs from P. (*P.*) *cheni* and *P.* (*P.*) *wui* from China, in having large, well-developed intestinal caeca, in the number and arrangement of caudal papillae.

The new form differs from *P.* (*P.*) *ardeae* in having alate spicules, *P.* (*P.*) *crassum* in having vulva anterior to mid of body, *P.* (*P.*) *depressum* and *P.* (*P.*) *indica* in the character of the anterior lobes of the lips in a pulp and in the number of caudal papillae. The new form has a close resemblance with *P.* (*P.*) *haliasturi* in having 5 pairs of postanal papillae, but however differs from it in having lateral alae, in having equal, alate spicules instead of subequal, non-alate spicules, in having 29 pairs of caudal papillae instead of 27 pairs and in having specimens of smaller size. Accordingly it is regarded as a new species with the specific name, *Porrocaccum* (*P.*) *accipiteri* sp. nov.

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Lucknow (India), *February* 3, 1977.

---

1. Baylis, H. A., *Nematoda, I,* 1936; p. 1.
2. Cram, E. B., *Bull. U.S. Nat. Mus.,* 1927, 140, 1.
3. Gupta, S. P., *Canad. Jour. Zool.,* 1960, 38, 315.
4. Hsu, H. F., *Parasit.,* 1933, 24, 512.
5. Subramaniam, G., *Zool. Anz.,* 1969, 182, 244.
6. Skrjabin, K. I., Shikobalova, N. and Mozyovoi, A. A., *Izdatelstvo–Akad. Nauk. S.S.S.R. Moscow,* 1951.
7. Yamaguti, S., *System Helminthum,* Parts I and II, 1961, 3, 1.

QUANTITATIVE DISTRIBUTION OF THE CARDIO-ACCELERATORY SUBSTANCE IN THE CENTRAL NERVOUS SYSTEM OF *HETEROMETRUS FULVIPES* C. KOCH, A SCORPION

AQUEOUS extracts of eserine treated portions of the Central Nervous System (CNS) of scorpion were tested for their acceleratory effect on isolated heart preparations of scorpion. Comparison of the effects with those of standard Acetylcholine (ACh) solutions, indicated that ACh-type substance was more abundant in the sub-oesophageal ganglion than in other portions of the CNS of scorpion.

Extracts from a variety of arthropod tissues[1-6] containing different substances have been observed to affect the heart rate. Acetylcholine (ACh)-like substance has been detected in the CNS of the scorpion and implicated in the regulation of heart rate[7], diurnal rhythmicity[8], locomotor activity[9] and electrical activity of the ventral nerve cord[8]. In the present investigation a study has been made of the distribution of ACh in various parts of the CNS of the scorpion and the effects of ACh and of extracts of CNS on heart activity are compared.

TABLE I

Distribution of ACh-like substance in the extracts of various parts of the CNS of the scorpion and effect of extracts expressed as per cent accelaeration over isolated heart of the scorpion

(*Values are mean and ± SD of 8 observations*)

Nature of the extract	ACh content (μg/g wet wt)	% Heart acceleration
Supra-oesophageal ganglion	23·10 ±2·86	20
Sub-oesophageal ganglion	41·26 ±5·12	44
Meso-somatic Nerve cord	14·24 ±5·66	14
Meta-somatic Nerve cord	20·26 ±2·05	17

The CNS of the scorpion *Heterometrus fulvipes* C. Koch, was isolated around 12·00 hrs of the day (as maximum ACh content was observed at this time[10]) in the cold room (10–15° C). For each observation, the CNS collected from at least 6 animals was divided into 4 extraction parts: the supra-oesophageal ganglion, the sub-oesophageal ganglion, the nerve cord with 3 meso-somatic ganglia and the nerve cord with the last 4 meta-somatic ganglia. ACh content was determined in different parts of the CNS by the Colorimetric method of Hestrin as given by Augustinsson[11]. Isolated parts of the CNS were treated with 1×10^{-5} M eserine for 5 min. in order to inactivate the enzyme AChE, responsible for the hydrolysis of ACh. One per cent (Wt/Vol) extracts were prepared in scorpion Ringer[12] and the homogenates were boiled and centrifuged. The supernatants were kept in the refrigerator (– 5° C) overnight and their effect on the heart of the scorpion isolated at 08·00 hrs next day (as minimum heart activity was observed during the period[13]) studied. The per cent change in heart rate over the control was calculated at the end of 5 min after the application of the individual extract or of standard solutions of ACh (E. Merck, Germany) of different concentrations. The amount of ACh present in the extracts of different parts of the CNS was calculated from the graph prepared with the standard amounts of ACh. Average of 8 readings was taken to represent the ACh content in each portion of CNS of the scorpion.

From the cardio-acceleratory effects of ACh and of extracts of the portions of the CNS of the scorpion, it was inferred that the ACh content was greater (41·26 + 1·2 μg/g wet wt.) in the suboesophageal region and least (14·24 + 5·66 μg/g wet wt.) in the meso-somatic region (Table I). Corresponding to the amount of ACh, the per cent acceleratory activity of the isolated heart of scorpion was also much greater when the extract of suboesophageal ganglion was added (44%) as compared to the addition of the extracts of other portions of the CNS (Table I). It is suggested that ACh-type of substance in the CNS of the scorpion may play a role in regulation of heart activity.

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---

1. Parrot, J. L., *Comp. Rend. Soc. Biol.*, 1941, 135, 929.
2. Maynard, D. M. and Maynard, E. A., *Gen. Comp. Endocrinot.*, 1962, 2, 12.
3. — and Welsh, J. H., *J. Physiol.*, 1959, 149, 215.
4. Alexandrowicz, J. S. and Carlisle, D. B., *J. Marine Biol. Assoc. (U.K.)*, 1953, 32, 175.
5. Cameron, M. L., *Nature (Lond.)*, 1953, 172, 349.
6. Rounds, H. D. and Garnder, P. E., *J. Insect Physiol.*, 1968, 14, 495.
7. Devarajulu Naidu, V., *Experientia,* 1969, 25, 1274.
8. Venkatachari, S. A. T. and Devarajulu Naidu, V., *Ibid.*, 1969, 25, 821.
9. Rao, K. P. and Gopalakrishna Reddy, T., *Nature (Lond.)*, 1967, 213, 1047.
10. Vasantha, N., Venkatachari, S. A. T., Murali Mohan, P. and Sasira Babu, K., *Experientia,* 1975, 31, 451.
11. Augustinsson, K. B., *Methods of Biochemical Analysis* (Ed. D. Glick, Inter Science Publishers Inc., New York, U.S.A.), 1957, 5, 1.
12. Padmanabhanaidu, B., *Nature (Lond.)*, 1967, 213, 410.
13. Devarajulu Naidu, V. and Padmanabhanaidu, B., *Indian J. Exp. Biol.,* 1976, 14, 1.

CIRCADIAN ACTIVITY OF ADENOSINETRIPHOSPHATASE IN THE NERVOUS SYSTEM OF THE COCKROACH, *PERIPLANETA AMERICANA*

The physiological changes in the activities of succinic dehydrogenase (SDH), phosphorylase and isocitrate dehydrogenase in the scorpions[1–3] and SDH and aminotransferase activities in cockroaches[4] were correlated with the locomotor activity of these animals. Adenosinetriphosphate (ATP) synthesizing system and enhanced biological oxidation of tricarboxylic acid cycle intermediates in rats[5] and ATP degrading system in hamsters[6] were also shown to be associated with their locomotor activity. The present study demonstrates the occurrence of a rhythmic variation of adenosinetriphosphatase (Mg^{2+} ATPase) in the nervous system of the cockroach, *Periplaneta americana*, under normal and constant light conditions.

Rearing of the cockroaches, etc., has already been described[7]. Under constant light (LL) and dark (DD) conditions, the animals were acclimatised for three months. For LL conditions, a light source was arranged 200 cm above the animal cage.

Mg^{2+} ATPase activity was determined according to Tirri *et al.*[8]. The content of inorganic phosphates was determined by the method of Fiske and Subba Row[9]. The enzyme activity was expressed as mg of Pi formed/g wet wt. of fresh tissue.

Mg^{2+} ATPase activity in the nervous system of normal (LD : 12 : 12 hours) animals exhibited cyclic variations with maximal activity at 00·00 h and minimal activity at 12·00 noon (Fig. 1 *a*). The average level of the enzyme activity was higher during 20·00 to 04·00 (dark hours) than during 08·00 to 16·00 (light hours) of the day (Fig. 1 *b*). Similar changes were reported in cholinesterases, dehydrogenases and aminotransferases in cockroaches[7,10,11] and scorpions[1–3]. Rhythmic variations in ATP were shown for vertebrates[5,6]. It is probable that enhanced ATPase activity during dark hours is related to the overt locomotor activity of the cockroaches, which are nocturnal in their habits[12].

Even under LL and DD conditions, persistence in the cyclic variations of ATPase activity was observed (Fig. 1 *a*). But the peak period of activity occurred at 04·00 hours, representing a phase shift by 4 hours over LD animals. Such phase shifts of about 4 hours are within the limits of the period range, *viz.*, 24 hours, of the enzyme activity.[13]

When mean enzyme activity for six (time) intervals under LL and DD conditions was compared with that of LD animals, it registered a loss of 16·2% and 10·0% respectively. This suggests that constant light rather than darkness exerts greater inhibitory effect on the enzyme activity. Moreover, in LL and DD conditions the average level of enzyme activity was

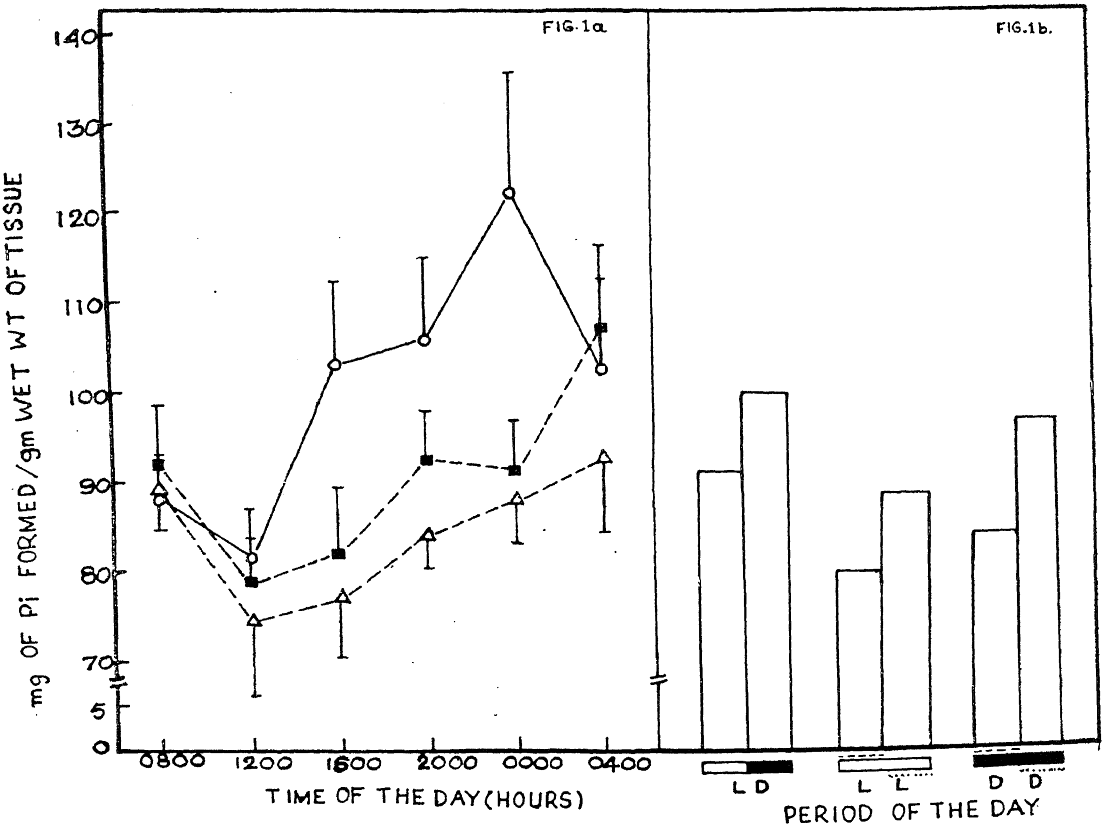

FIG. 1 *a–b*. Fig. 1 *a*. Rhythmic ATPase activity in the nervous system of the cockroach, *Periplaneta americana*, under LD (normal) (○———○), continuous light (LL) (△- - - - -△) and dark (DD) (■- - - - -■) conditions. The enzyme activity was expressed as mgm of Pi formed/gm wet weight of fresh tissue. Total number of animals used were 18 per each experiment. The values are average of four experiments. Fig. 1 *b*. Average level of enzyme (Mgz^{+} ATPase) activity during 20·00 to 04·00 hours (▬ for LD and ▭, ▬ for LL and DD conditions respectively) and 08·00 to 16·00 hours (▭, ▭, ▬ fcr LD LL and DD conditions respectively). The enzyme activity was expressed as mgm of Pi formed/gm wet weight of fresh tissue. Total number of animals used were 18 per each experiment. The values are average of four experiments.

high during 20·00 to 04·00 hours than during 08·00 to 16·00 hours (Fig. 1 *b*). This would mean that the animals, even under constant conditions continue to maintain its original pattern of enzyme activity. Thus, the periodic oscillations are independent of the environmental light conditions (LL and DD), implying the endogenous nature of the rhythm as suggested by Brady.[14]

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---

1. Devarajulu Naidu, V., *Doctoral Dissertation*, S.V. University, Tirupati (India), 1973.
2. Chandrasekhara Reddy, D. and Padmanabha Naidu, B., *Curr. Sci.*, 1977, 46, 147.
3. Mastanaiah, S., Changalraju, D. and Swami, K. S., *Experientia*, 1977 (In press).
4. Vijayalakshmi, S., *Doctoral Dissertation*, S.V., University, Tirupati (India), 1977.
5. Glick, J. L. and Cohen, W. D., *Science*, 1964, **143**, 1184.
6. Nishiitsuje-Uwo, J. Townsend, R. N., Nakajima, H. and Pittendrigh, C. S., *Comp. Biochem. Physiol.*, 1961, **22**, 319.

7. Vijayalakshmi, S., Murali Mohan, P. and Sasira Babu, K., *J. Ins. Physiol.*, 1977, **23**, 195.
8. Tirri, R., Lagerspetz, K. Y. H. and Kohonen, J., *Comp. Biochem. Physiol.*, 1973, **44B**, 473.
9. Fiske, C. H. and Subba Row, Y., *J. Biol. Chem.*, 1925, **66**, 375.
10. Vijayalakshmi, S., Pavan Kumar, I., Murali Mohan, P. and Sasira Babu, K., *Comp. Physiol. Ecol.*, 1977, **2** (2), 58.
11. —, and Sasira Babu, K., *Abstract 2nd All India Symposium on Comparative Animal Physiology*, December 13 to 15, 1976, Nagpur (India).
12. Harker, J. E., *Nature* (*Lond.*), 1954, **173**, 689.
13. —, *The Physiology of Diurnal Rhythms* (Eds.) T. A. Bennett-Clark, George Salt, V. B. Wigglesworth), *Cambridge Monographs in Experimental Biology*, 1964, No. 13.
14. Brady, J., *Adv. Ins. Physiol.*, 1974, 10, 1.

MIXED-COMMUNAL ROOSTING OF INDIAN WHITE BACKED VULTURES IN POONA

WHILE observing the roosting behaviour of some common birds of Poona (Maharashtra) and around, from January 1976 to February 1977, I came across number of mixed-communal roosts of birds.

Usually the associates which I have observed at such roosts are Indian Mynas, *Acridotheres tristis* (Linn.); House and Jungle Crows, *Corvus splendens* Vieillot and *C. macrorhynchos* Wagler; Pariah Kites, *Milvus migrans* (Boddaert); Rose ringed Parakeets, *Psittacula krameri* (Scopoli) and House Sparrows, *Passer domesticus* (Linn.). These associations at mixed-communal roosts are noticed throughout the year. The Cattle Egrets, *Bubulcus ibis coromandus* (Boddaert) and Indian Pond Herons, *Ardeloa grayii grayii* (Sykes) also assemble at such mixed roosts only during the non-breeding season while a migratory bird like Rosy Pastor, *Sturnus roseus* (Linn.) roosts together along with the above species for a part of the year. This has also been indicated by Gadgil and Ali[1] (1975) who have given a systematic account of communal roosting of Indian birds.

During the month of June 1976, it was noticed for the first time that the Indian Whitebacked or Bengal Vultures, *Gyps bengalensis* (Gmelin) formed a mixed-communal roost along with the Indian Mynas, House and Jungle Crows and Whitenecked Storks, *Ciconia episcopus* (Boddaert). This mixed congregation was observed on the Poona–Bombay Road near Dapodi, on a Banyan tree, *Ficus bengalensis* L. On counting the number of birds at this mixed roost, it was found that there were 150 Indian Whitebacked Vultures, 300 Indian Mynas, about 150 House and Jungle Crows and a pair of Whitenecked Stork. These countings were made in the evening, when these birds return to the roost. Further observations showed that this mixed-communal roost was constant till september 1976, both in the number of species and in their total population. Subsequently, the roost totally disappeared till February 1977. In the meantime, the Vultures and Storks seem to have migrated from this locality, while Mynas and Crows shifted to another roost about half a kilometer away from the original roosting place.

From the above observations it would appear that Indian Whitebacked Vultures are seen on this mixed-communal roost only during the monsoon from June—September, which is a part of their non-breeding season.

I take this opportunity to thank, Dr. B. K. Tikader, Deputy Director, Zoological Survey of India, Poona, for his co-operation.

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1. Gadgil Madhav and Salim Ali, *J. Bombay Nat. Hist. Soc.*, 1975, 72 (3), 716.

NEW PARASITES RECORDED ON THE SORGHUM SHOOTFLY, *ATHERIGONA SOCCATA* (RONDANI)

VARIOUS cultural and chemical control methods have been investigated and recommended for the control of shootfly (*A. soccata*), a serious pest of sorghum. However, very little work has been done on identifying and utilizing the natural enemies of this pest. This aspect is now being systematically investigated under the All India Co-ordinated Sorghum Improvement Project at New Delhi.

One to two week old shootfly infested seedlings, having freshly formed dead hearts, were collected from the field and were kept in separate glass jars containing 5 cm layer of moist sand. The number of dead hearts with the shootfly larvae were counted in each jar to determine the percentage of parasitism. The jars were placed at 27° C ± 1° C, the relative humidity ranged between 60 and 70%.

The parasites and shootfly adults emerging in jars were periodically collected and sent to British Museum for identification. It is found that in addition to the two parasites already recorded, *viz.*, *Aprostocetus* sp. and *Callitula bipartitus*

(Kundu *et al.* 1971)[1], the shootfly larvae were also parasitised by *Ganaspis* sp. (Eucoilidae), *Psilus* sp. (Diapriidae), *Hemiptarsenus* sp. (Eulophidae) and *Diaulinopsis* sp. (Eulophidae).

The observation on the percentage of parasitism taken during different periods showed that except for 2% parasitism by *Ganaspis* sp. in the month of April, 1975, these parasites were recorded only in the infested seedlings collected during the months of September and October. Even in these months the extent of parasitism was rather low and ranged from 1 to 4%. The two parasites recorded earlier, *viz. Aprostocetus* sp. and *Callitula bipartitus* were reared out in the month of August also. The percentage parasitism of *Aprostocetus* sp. was found to be higher than any other parasite and ranged from 4% (October) to 15% (September).

Perusal of literature showed that these parasites have been recorded from a number of dipterous hosts from different parts of the world. Kerrich (1962)[2] reported *Hemiptarsenus semialbiclavus* (Gir.) parasitizing Agromyzid leaf miners of vegetable crops in Africa, while *Psilus* sp. has been recorded as pupal parasite of *Pholeomyia comans* (Diptera : Milichiidae) by Mosev and Neff (1972)[3]. *Diaulinopsis* sp. was rared from *Agromyza pusilla* Mg. (Diptera : Agromyzidae) from North America, U.S.A. (Thompson 1955)[4]. *Eucoila (Ganaspis) haywardi.* Blanch. has been used for the control of fruit flies, *Anasterpha* spp. (Diptera : Tephritidae) in Argentina (Turica 1968)[5]. The sorghum shootfly (*A. soccata*) has not so far been recorded as host of these parasites and thus constitutes a new host record. These four parasites also constitute first record from India.

The authors are grateful to the Director, Commonwealth Institute of Entomology, London, for identification of the parasites and to Dr. N. C. Pant, Head of the Division of Entomology, for providing the necessary facilities. Thanks are also due to Dr. S. I. Farooqi, for going through the manuscript.

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---

1. Kundu, G. G., Prem Kishore and Jotwani, M. G., *Investigations on Insect Pests of Sorghum and Millets,* Final Technical Report, Division of Entomology, IARI, New Delhi, 1971, p. 145.
2. Kerrich, G. J., *Bull. ent. Res.,* London, 1969, 59 (2), 195.
3. Mosev, J. C. and Neff, S. E., *Z. angew* 1972, 69 (4), 343.
4. Thompson, W. R., *Parasitic Catalogue* 2, Part 3—Host of Hymenoptera ((ratid to Evaniid), 1955.
5. Turica, A., *Lucha Biologica como me control de Las moscas de Los* (Biological control as a means fc control of fruit flies), India, 1968, N p. 29 [*RAE,* 57 A, 1820].

CYANOPHAGE AC-1 INFECTING THE Bl GREEN ALGA *ANACYSTIS NIDULAN*

AS–1 TYPE cyanophage which infects the cellular algae, *Anacystis nidulans* and *Synecho cedrorum* was first characterized by Safferman It is the largest BGA phage so far examine a head diameter of 90 nm. and tail-head ra 3 to 1, based on a tail length of 243·5 nm. a rigid tail with a contractile sheath and plate with tail pins. In the present report, phage type infecting *Anacystis nidulans* 1401 *Chroococcus minor* ARM was isolated fr waste stabilization pond inside the campus Indian Agricultural Research Institute, New I

The phage formed clear plaques of 4– after 10 days of incubation. Several blue algal species of *Nostoc, Anabaena, Tolyp Aulosira* and *Spirulina,* the green alga *Ch vulgaris* and the bacteria, *Azotobacter chrooc Rhizobium* spp. and *Rhodopseudomonas caps* were also tested for susceptibility to this but none of them was found susceptible.

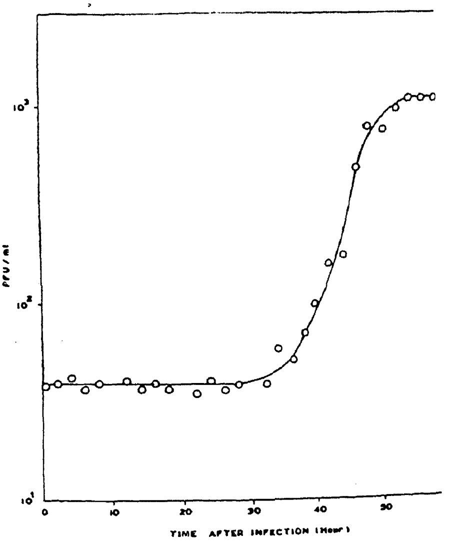

FIG. 1. One step growth curve of AC–1 phage.

The phage titer was stable in its own lysate and was rapidly inactivated at high temperatures. The adsorption of the phage followed the kinetics of a first order reaction and about 95% of the phage particles was adsorbed to the host cells after 40 min in stationary cultures. The one step growth curve of the phage resembled that of SM–1[3], with a latent period of 30 h followed by a rise period of 20 h (Fig. 1), with an average burst size of about 30 PFU/infected cell.

Electron micrographs of the preparation revealed phage-like particles with a polyhedral head and a tail. The polyhedral head was 62·5 nm diameter with a tail head ratio of 1 : 3, based on the tail length of 25 nm (Fig. 2). The short non-contractile tail of AC–1 differentiates it from AS–1 and brings it closer to SM–1[4].

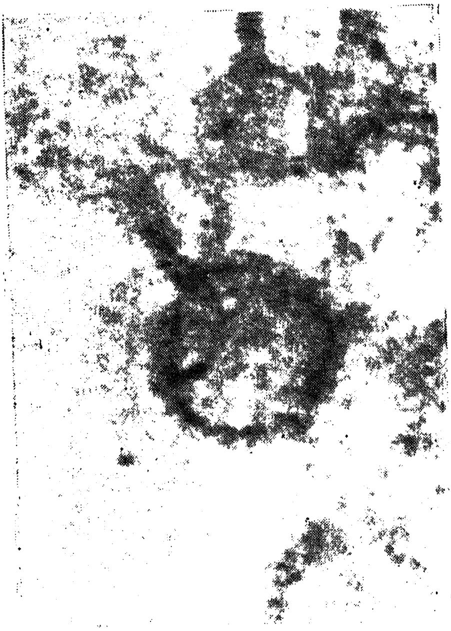

FIG. 2. AC–1 cyanophage particles (× 394,000).

AS–1 infects *Anacystis* and *Synechococus*[1]. SM–1 infects *Synechococcus* and *Microcystis*[5] and the present AC–1 infects *Anacystis* and *Chroococcus*. If the taxonomic merger of *Anacystis* with *Synechococcus* is accepted as suggested by Padmaja and Desikachary[6], the genus *Synechococcus* appears to have three types of phages (SM–1, AS–1 and AC–1) infecting it.

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---

1. Safferman, R. S., Diener, T. O., Desjardins, P. R. and Morris, M. E., *Virology*, 1972, 47, 105.
2. Venkataraman, G. S., Kaushik, B. D., Subramanian, G., Shanmugasundaram, S. and Govindarajan, A., *Curr. Sci.*, 1973, 42, 104.
3. Mackenzie, J. J. and Haselkorn, R., *Virology*, 1972, 49, 505.
4. — and —, *Ibid.*, 1972, 49, 497.
5. Safferman, R. S., Schneider, I. R., Steere, R. L., Morris, M. E. and Diener, T. O., *Ibid.*, 1969, 37, 387.
6. Padmaja, T. D. and Desikachary, T. V., *Phykos*, 1968, 7, 62.

ASSOCIATION OF SOME LEAF CHARACTERS WITH PHOTOSYNTHESIS IN RICE

PHOTOSYNTHETIC process as a limiting factor for crop yield is receiving considerable attention in recent years. Differences in photosynthetic rates among cultivars of various crop species including rice have been reported[1-3]. But a correlation between photosynthetic rate per unit leaf area and economic yield has not always been found within a species[4]. However, the information on variability in photosynthetic rate is necessary for selection of photosynthetically efficient genotypes, as a first step, if enhanced photosynthesis can influence the crop yield[2]. Leaf characters associated with photosynthetic rates of cultivars are of obvious importance for rapid measurement in field selection programme[1]. In the present study, the association of some of the leaf characters with photosynthetic rate in rice was assessed with a view to define more appropriate indices for selection.

Cultivars belonging to early and late maturity groups of tall *indicas* as well as high yielding group (HYV) were studied with 16 entries in each group. Leaf chlorophyll content[5], stomatal frequency[6] and size[7], interveinal distance[8], specific leaf weight (SLW)[9] and total leaf nitrogen content per unit area (N_{LA})[3] were assessed at peak vegetative stage using standard procedures. Photosynthetic rates (Po) were computed as described elsewhere[10], with the exception that excised single leaves were fed with radioactive carbon ($Na_2{}^{14}CO_3$, specific activity of 47·6 mCi.mM^{-1}). The cpm values were converted to mg CO_2.dm^{-2}hr^{-1} adopting the formula suggested by Naylor and Teare[11].

Considerable variation in all the above characters including Po was observed. Among the varieties tested, *Ptb* 10, *Co.*13 (early), *Peta, Mahsuri* (late); *Padma* and *Cauvery* (HYV) recorded high Po (about 50 mg).

TABLE I

Relationship between photosynthetic rate (Po) and some leaf characters in rice

Leaf character	Correlation with Po (r)*			
	Early	Late	HYV	Pooled value
Chlorophyll (mg. dm^{-2})	0·574*	0·822**	0·781**	0·742**
Stomatal frequency (number mm^{-2})	0·536*	0·561*	0·553*	0·550*
Stomatal size ($b \times n \times 10^{-3}\,\mu cm^{-2}$)	0·543*	0·607*	0·529*	0·561*
Interveinal distance (μ)	−0·826**	−0·723**	−0·603*	−0·727**
Leaf nitrogen content N_{LA} (mg · dm^{-2})	0·554*	0·650**	0·806**	0·685**
Specific leaf weight, SLW (mg · dm^{-2})	0·578*	0·634**	0·687**	0·635**

Significant at 1% (**) and 5% (*) level.

Correlation tests (Table I) showed significant relationship of Po with all the leaf characters studied especially with that of chlorophyll content (+ ve) and interveinal distance (− ve). The relationship between Po and chlorophyll has already been fairly established[12]. However, such relationship should be viewed with caution as the chlorophyll content though high sharply affects the photosynthetic rates under limited light conditions[13]. The N_{LA} also showed high association with Po and corroborated similar findings elsewhere[3]. However, it has been observed that P and K deficient rice plants were poorer in Po in spite of sufficient N in their leaves[14]. Specific leaf weight or the leaf thickness also showed high relationship with Po as has been reported earlier[15]. However, such relationship was found to be not consistent by some workers[3]. Among the characters studied, stomatal frequency and size showed loose relationship with Po in all the groups. In general, the relationship of interveinal distance with Po is consistent among all the groups. It is suggested that smaller distance between veins might help in efficient transport of photosynthates and enhance the rate of CO_2 fixation[8]..

Thus, the interveinal distance might serve as a simple and reliable index in identifying photosynthetically efficient genotypes. So far, the relationship of smaller interveinal distance with photosynthetic efficiency in rice has not been reported.

The authors are grateful to the Department of Atomic Energy, Government of India, for financial assistance to the above investigations.

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1. Irvine, J. E., *Crop Sci.*, 1975, 15, 671.
2. Krenzer, (Jr.), E. G. and Moss, D. N., *Ibid.*, 1975, 15, 71.
3. Tsunoda, S., In : *IRRI Philippines, Rice Breeding*, 1972, p. 471.
4. Asana, R. D., *Indian J. Pl. Physiol.*, 1968, 11, 1.
5. Arnon, D. I., *Pl. Physiol.*, 1949, 24, 1.
6. Mohan Ram, H. Y. and Nayyar, V. L., *Stain Technol.*, 1974, 49, 114.
7. El Sharkawy and Hesketh, J. D., *Crop Sci.*, 1964, 4, 619.
8. Crookston, R. K. and Moss, D. N., *Ibid.*, 1974, 14, 123.
9. Yoshida, S., Forno, D. A., Cock, J. H. and Gomez, K. A., *Laboratory Manual for Physiological Studies of Rice*, IRRI, Philippines.
10. Murty, K. S., *Indian J. Pl. Physiol.*, 1969, 12, 202.
11. Naylor, D. G. and Teare, I. D., *Agron. J.*, 1975, 67, 404.
12. Murata, Y., In : *Physiological Aspects of Crop Yield* (Eds. Eastin, J. D. *et al.*), ASA and CSSA, 1969, p. 235.
13. Kariya, K. and Tsunoda, S., *Tohuku J. agr. Res.*, 1972, 23, 1.
14. Watanabe, H. and Yoshida, S., *Soil Sci. Pl. Nutr.*, 1970, 16, 62.
15. Murty, K. S., Nayak, S. K., Sahu; G.; Ramakrishnayya, G., Janardhan, K. V. and Rai, R. S. V., *Pl. Biochem. J.*, 1976, 3, 63.

TRITICALE STEM RUST FROM INDIA

Triticale is a new plant genus produced artificially by crossing either hexaploid or tetraploid wheat (*Triticum* sp.) with diploid rye species (*Secale* sp.) followed by the doubling of the chromosome complement of the sterile F_1 hybrid. It is generally believed that wheat is resistant to the rust forms which attack rye, and that rye is not attacked by rusts specialized to wheat[3]. As a result *triticale* could be expected to be resistant to both rust forms. But this epectation has not come true and the cereal rusts (*Puccinia* spp.) are observed on triticale and are considered to be the most serious threat to its production. A lot of work is being done for the improvement of this crop. Recently, during April, 1977 a severe attack of stem rust was noticed on indigenous (TL–29 and TL–193) and Mexican (CIMMYT) material in the Experiemental Farm of Punjab Agricultural University, Ludhiana. The disease incidence in the material was as high as 80 to 100%.

The characteristic symptoms observed on triticale were small to large, brown pustules on the stem, leaf sheath (Fig. 1) and leaves. In the highly susceptible material, ears were also attacked. Uredospores were variable in shape ranging from round, oblong, ellipsoid or elongated, thick walled, golden brown, strongly echinulated, 24–53·5 × 14·5–26·8 μ. Teleutospores ellipsoid, oblong, bicelled, constricted at the septum, wall thickened at the apex, smooth, dark brown, 15–24·5 × 35–60 μ. The fungus is identified as *Puccinia graminis f.* sp. *tritici* (Pers) Eriks & Henn.

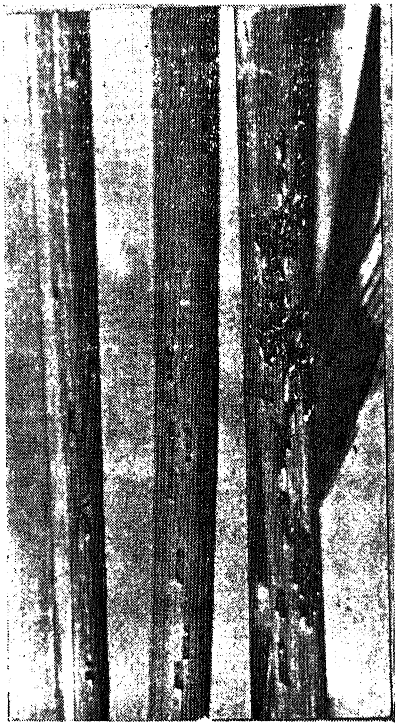

FIG. 1. Rust pustules on triticale.

The triticale (TL–29) and wheat (agra local) were inoculated with freshly collected uredospores from triticale. Ten-day old plants after inoculation were kept in a humidity chamber for 24 hrs. and were transferred to glass house at 25° C. The characteristic symptoms were observed both on triticale as well as on wheat plants after 15 days of inoculations.

Stem rust has already been reported on *Triticale* in Mexico[1] and Czechoslovakia[2], but from India it has not been reported so far. Its occurrence and the future potential threat, in India, should also be taken into consideration in the breeding programmes.

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---

1. Lopez, B. A., *M.Sc. Thesis,* Mexico, 1971, p. 60.
2. Rimen, L., *Referativnyi Zhurnal,* 1974, **55**, 1194.
3. Watson, I. A. and Luig, N. H., *Proc. 3rd Int. Wheat Genet. Symp. Caneberra,* 1968, p. 227.

ON THE OCCURRENCE OF ALTERNATIVE GERMINATION IN THE GENUS *PHASEOLUS* L (PAPILIONACEAE)

THE importance of seedling characters have lately been realized and it is only relatively recently that information on seedling characters began to be used for identification purposes[1,4,5] and even for genetic studies[2].

Seedlings characters in the Leguminosae have been studied by Burger[1] and Kalyansundaram[4]. The latter author surveyed 58 genera and 200 species for the germination character and has shown the occurrence of both types of germination in Papilionaceae and Caesalpinae. In the former, hypogeal germination being more common than the epigeal, and the reverse in the latter, while Mimosae shows epigeal type of germination only.

In this communication, the authors report the occurrence of alternative germination in the genus *Phaseolus* whose epigeal germination is well known.

In Table I data pertaining to different species of *Phaseolus* investigated with regard to the type of germination are listed. Out of the 9 species investigated *P. angularis* and *P. multiflorus* show hypogeal germination and *Phaseolus vulgaris* (Figs. 1–3) as also the six other species show epigeal germination. It may be further noted that even the large number of seedlings tested under the different varieties of *P. mungo*, *P. vulgaris* and *P. aureus* included in the present study show only epigeal type of germination though transitional forms are known to occur[3]. To find out whether the epigeal

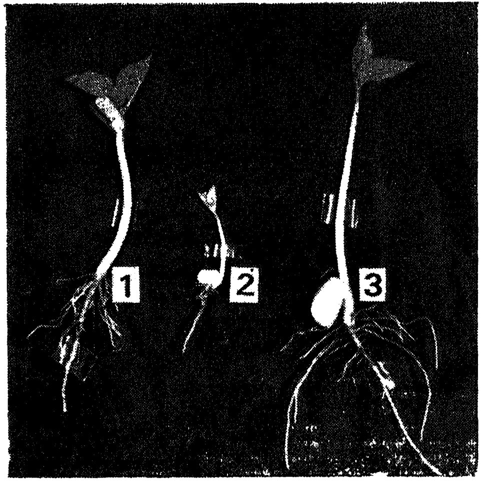
TABLE I

List of Phaseolus *species showing type of germination*

Sl. No.	Species/Variety	Type of germination	Number of seedlings tested	Source of material
1.	*Phaseolus angularis* EC 87897	Hypogeal	113	N.B.P.I., Reg. Stn., Simla
2.	*Phaseolus multiflorus* PLB 190–2	do.	99	do.
3.	*Phaseolus lunatus* EC 18199	Epigeael	102	do.
4.	*Phaseolus vulgaris* PLB 14·1	do.	83	do.
	Phaseolus vulgaris PLB K 1	do.	78	do.
	Phaseolus vulgaris (Local)	do.	109	Agric. Market, Warangal
	Phaseolus vulgaris Premier	do.	59	Commercial, Hyderabad
5.	*Phaseolus aureus* (Local)	do.	2142	Agric. Res. Stn., Warangal
	Phaseolus aureus PLM 629	do.	35	D.P.I., IARI, New Delhi
	Phaseolus aureus Pusa Baisakhi 100	do.	102	Commercial, Hyderabad
	Phaseolus aureus- Von G 65	do.	63	Agric. Res. Inst., Hyderabad
	Phaseolus aureus PR–16	do.	131	Commercial, Hyderabad
6.	*Phaseolus mango* PLM–221	do.	28	D.P.I., IARI, New Delhi
	Phaseolus mungo (Local)	do.	2980	Agric. Market, Warangal
	Phaseolus mungo U–30	do.	45	A.R.I., Hyderabad
	Phaseolus mungo US–132	do.	64	do.
	Phaseolus mungo Krishna–11	do.	102	do.
7.	*Phaseolus atroperpolius* EC 276115	do.	16	D.P.I., IARI, New Delhi
8.	*Phaseolus aconitifolius* (Local)	do.	22	Kakatiya University Campus, Warangal
9.	*Phaseolus trilobus* (Local)	do.	23	do.

and hypogeal type of germination has any functional significance to the species, we looked for the presence of stomata on the flat, smooth, inner and outer convex surfaces of the cotyledons. The outer surface of the cotyledons of the epigeal seedlings was found to be stomatiferous while the stomata were totally absent in hypogeal cotyledons. The inner smooth surface in both the epi- and hypogeal seedlings was devoid of stomata and the epidermis was made of rectangular cells in the former and elongated to polygonal cells in the latter. The mature stomata are anomocytic with 5–6 surrounding cells and measure $14 \cdot 5 \times 10 \cdot 5\,\mu$ with an ellipitic stomatal pore. The guard cells are typically kidney shaped with their inner walls thicker than the outer and with several chloroplasts in them and hence functional. Yet another interesting observation is that the epigeal cotyledons are rough and wrinkled with linear striations externally while the hypogeal cotyledons are smooth. We are unable to offer any explanation for this behaviour but it is probable that it may be functionally related to the stomatiferous epigeal cotyledons.

Kalyansundaram[4] regards hypogeal germination in the Leguminosae as ancestral and the epigeal as derived from it. But according to Eames[5] hypogeal germination is 'clearly advanced' and epigeal germination primitive. The occurrence of alternative type of germination just described, to our knowledge, is not on record for the genus *Phaseolus*. In view of the controversial opinions expressed the present observations are significant and may serve as taxonomic pointer in delimiting species. The occurrence of alternative germination in the genus *Phaseolus* thus raises phyletic problems and until further work is carried out it would be premature to regard a given *Phaseolus* species as primitive or advanced merely on the germination criteria alone.



FIGS. 1–3. Fig. 1. *Phaseolus vulgaris* showing epigeal seedlings. Figs. 2–3. *P. angularis* and *P. multiflorus* showing hypogeal seedlings.
N.B. Longitudnal striations on cotyledons of epigeal cotyledon can be seen while the hypogeal cotyledons are smooth.

We are grateful to the Directors of the various Agricultural Research Stations, for kindly providing the seed material of various species investigated and to Prof. U. B. S. Swami, for giving us facilities and encouragement.

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---

1. Burger, Hzn, D., *Seedlings of Some Tropical Trees and Shrubs Mainly of S.E. Asia*, Translated from Dutch by G. Burgan and S. Festing, Centre for Agric. Publ. and Documentation, Wageningen, 1972.
2. Crow, L. K., "Seedling characters," In : *Teaching Genetics*, Eds., C. D. Darlington and A. D. Bradshaw, Oliver and Boyd, London, 1966.
3. Eames, A. J., *Morphology of the Angiosperms*, McGraw-Hill, N.Y., 1961.
4. Kalyansundaram, S., "On the hypogeal and epigeal germination of seeds in the family Leguminosae," *Proc. 45th Indian Sci. Congr., Part III*, 1958. p. 308. (Abstract).
5. Koller, D., "Germination," *Sci. Amer.*, 1959, 200 (4), 75.

AN IMPROVED AND SIMPLE LEUCOCYTE CULTURE TECHNIQUE FOR CHROMOSOMAL PREPARATIONS FROM ALBINO RAT

RODENTS have been widely used for many biomedical investigations but their use for cytogenetic studies is not so extensive. One reason may be the lack of reliable tissue culture techniques, *in vitro*, for yielding a large number of cells at metaphase. The media for leucocyte cultures described by Nichols and Levan[1,2] (1961, 1962) and Ford and Woollam[3] (1963) have led to hemolytic reactions in our laboratory resulting in poor cell growth. The new method developed in this laboratory is a modification of the techniques described by William and Ray[4] (1965) and Sankar and Giesler[5] (1973). The technique reported here has certain advantages over existing ones. The medium, TC 199 has been employed in place of other media and Phytohemagglutinin–P, a more powerful agglutinating agent, has been added to the medium instead of Phytohemagglutinin–M. This has resulted in a considerable increase in the number of cells at metaphase and as a result, a minimum of 10 slides can be prepared from each tube. Furthermore the use of diluted foetal calf serum and very dilute sodium citrate, for hypotonic treatment[5], has been omitted. Treatment with Hank's solution followed by a brief incubation in 1% sodium citrate has been used instead. Another advantage of this technique is its adaptability, by suitable modifications, for culturing leucocytes from other rodents as well. For culturing leucocytes, the whole blood can also be used without going through the procedure of separating leucocytes but we prefer leucocyte separation for obtaining optimum cultures. The minimum mitotic index obtained was 30.

The blood was drawn into a 10 ml sterilized syringe, by cardiac puncture, rinsed with heparin. 1 ml of plasma containing $1 \cdot 2 \times 10^6$ leucocytes/ml was added to the culture tubes (Corning, 30 ml) having the prewarmed (37° C) culture medium. The medium included TC Medium 199 (Difco) 10 ml ; 1 ml of a solution of Penicillin–G (1000 units) and Streptomycin sulfate (1000 mcg) (Sigma) ; 1·80 ml L-Glutamine (Sigma) 100 μ moles/ml in saline ; 0·02 ml of Phytohemagglutinin-P (Difco) and 200 I.U. Heparin (Koch Light Laboratories). The cultures were incubated for

72 hours at 37° C. The pH of the medium was kept between 7·5 and 8·0 during the incubation period by loosening the cap by 1/3 turn daily.

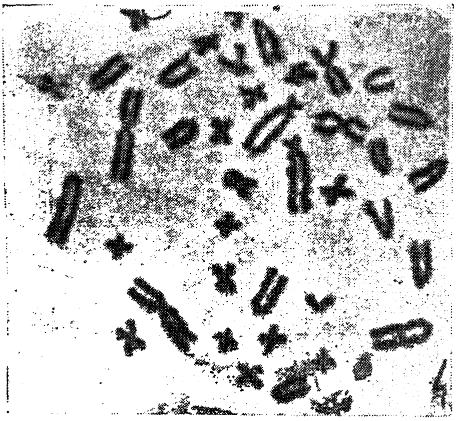

FIG. 1. Metaphase chromosomes from albino rat leucocytes cultured *in vitro*, (× 2,300) photographed on a Kodak Micro-File (panchromatic) Film using Carl Zeiss 'Ergavil' Research Microscope with Automatic Photomicrography Equipment.

0·2 ml of Colchicine (4 mcg/ml, Roussel Uclaf) was added to the tubes 2 hours prior to the harvesting of the cells. The tubes were then centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and the cells were suspended in 5 ml of prewarmed (37° C) Hanks solution. The tubes were again centrifuged at 1000 rpm for 5 minutes. The supernatant was aspirated with a pippet. The cells were resuspended in 3·5 ml of prewarmed (37° C) 1% sodium citrate solution and the suspension was incubated at 37° C for 10 minutes and then centrifuged at 800 rpm for 6 minutes and the supernatant was discarded. The cells were resuspended in 1 ml of cold acetic acid-methanol fixative, for each ml of the original solution, for 10 minutes at room temperature. This suspension was then centrifuged at 800 rpm and 2 more changes of the fixative, after 15 minutes in each, were given. The cells were finally suspended in 1·5 ml of the fixative. The slides were prepared by the flame drying technique and the preparations were stained with phosphate buffered Giemsa stain, pH 6·8.

Results

Sufficient number of metaphase spreads were obtained for each slide prepared by this technique for either karyotyping or chromosomal analyses by banding pattern technique. Over 125 cultures have been established in this way and quite satisfactory preparations were obtained for chromosomal study (Fig. 1).

The authors are extremely grateful to Prof. and Mrs. S. Hurtig (France) for providing TC Medium 199 and Phytohemagglutinin-P as a gift. Sincere thanks are also expressed to Head, Department of Zoology, for providing necessary laboratory facilities.

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---

1. Nichols, W. W. and Levan, A., *Blood Bone Marrow Tissue Culture and Cell Separation Conf.*, Bethesda, Maryland, October 20-21, 1961, p. 1.
2. — and —, *Blood*, 1962, 20, 106.
3. Ford, E. H. R. and Woollam, D. H. M. *Stain Technol.*, 1963, 38, 271.
4. Williams, T. W. and Ray, M., *Cytogenetics* 1965, 4, 365.
5. Sankar, D. V. Siva, and Gieseler, A., *Cytologia* 1973, 38, 155.

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BETA SPECTRUM OF ^{143}Ce

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ABSTRACT

The beta spectrum of the 37 hour negatron decay of ^{143}Ce has been analysed employing a Siegbahn-Slatis beta ray spectrometer and slow-fast coincidence system using sources from enriched ^{142}Ce samples for exact end point energies, intensities and log ft values of different beta groups. The results are incorporated in a decay scheme.

INTRODUCTION

THE negatron decay of the 33hr. ^{143}Ce was extensively studied and the prominent levels of ^{143}Pr were well established. However, there has been no detailed beta spectrum analysis of the ^{143}Ce isotope. The reported information on the beta groups feeding different levels in ^{143}Pr, their end point energies, intensities and log ft values are only from the gamma-spectroscopic investigations. Gopinathan[1] reported the end point energies of the two intense beta groups feeding the 57 keV and the 351 keV levels in ^{143}Pr as 1400 keV and 1110 keV. Megli *et al*[2] obtained from their gamma-spectroscopic data, the values of 1380 and 1093 keV for the two intense beta groups. Sunyar's[3] analysis of their gamma-spectral data gives 1408 and 1174 keV for the above two beta groups. Thus even in the available data on the end point energies of the most intense beta groups, as can be seen from Table I, there is a vast disparity. Such is also the case with their intensities and log ft values. The reported results on the intensity of the outermost beta group is 38·4% according to Gopinathan *et al*[1], 29·0% as per Megli *et al*[2] and 51·05% due to Sunyar *et al*[3]. Similarly in the case of the beta feeding, the 351 keV level of ^{143}Pr, the intensities are widely varying, *viz.*, 41·5% as per Gopinathan *et al*[1], 53·0% due to Megli *et al*[2] and 36·94% according to Sunyar[3].

Hence a detailed analysis of the beta spectrum of ^{143}Ce has been carried out both in the singles and coincidence studies in order to get complete data on the exact end point energies, intensities and log ft values of the different beta groups.

EXPERIMENTAL DETAILS AND RESULTS

Enriched ^{142}Ce stable isotope with 92·77% enrichment is obtained from Oak Ridge National Laboratory, Tennessee, U.S.A. in the form of cerium oxide. ^{142}Ce (25 mg.) is irradiated at the CIRUS reactor of Bhabha Atomic Research Centre, Bombay, each time getting the required activity of the ^{143}Ce isotope. Three such irradiations have been carried out. Sources are obtained as cerium chloride in dilute hydrochloric acid, in three consignments. Sources are prepared on mylar foils of thickness 150 μg/cm^2. Insulin is used to define the perfect source diameter. Typical source diameters are 2 mm.

The most intense 294 keV gamma is fixed on the gamma channel of the Siegbahn-Slatis beta spectrometer described by Nagarajan *et al*[4] and suitably modified[5] for beta-gamma coincidence studies. The resolution of the gamma detector [NaI(Tl)] employed is 10% for the 661 keV gamma rays. The beta spectrum in coincidence with the 294 keV gamma is scanned in steps of roughly 25 keV from 750 keV onwards to avoid the interference from inner beta-gamma cascades. The count rate is corrected for halflife and for beta-gamma angular correlation due to Raju *et al*[6]. The resolving time of the coincidence circuit is reduced to 2 T = 0·03 μ sec to improve the true to chance coincidence ratio, particularly near the end point energy of the beta spectrum. The coincidence spectra are recorded repeatedly. The Fermi-Kurie plot after its correction for any shape deviation has resulted an end-point energy of 1110 ± 2 keV from a computer program.

In the singles mode of operation of the spectrometer, the beta spectrum is scanned down from 200 keV to the end point energy of the highest energy beta group in steps of 20 keV. The half-life corrected spectrum is used for the analysis into different beta groups. Using the spectral shape[5] of the outermost beta group, the spectrum is corrected for its shape for the 1404 keV beta group. The shape corrected spectrum is then subtracted from the total beta spectrum. This resulting spectrum is again corrected for the small order shape deviation[7] of the second beta group with an end-point energy of 1110 keV obtained from the coincidence measurements. This resulted

TABLE I

Comparison of beta spectral data due to different authors

Beta Transition	Present work			Gopinathan *et al.*			Sunyar *et al.*			Megli *et al.*		
	Energy (keV)	%	log ft.	Energy (keV)	%	log ft.	Energy (keV)	%	log ft.	Energy (keV)	%	log ft.
	..	..	..	65	0·2	5·5	41	0·02	5·6	..	..	..
	..	..	..	..	..	..	58	0·05	5·8	83	0·02	6·8
	..	..	..	300	1	6·0	279	0·6	7·0	307	0·39	7·4
	405±7	..	..	..	..	..	378	0·1	8·2	404	0·07	8·5
	515±5	2·5	7·5	520	2·6	7·3	502	1·7	7·4	528	1·15	7·5
	740±4	10	7·0	735	6·2	7·0	710	16	7·0	743	10·3	7·2
	..	..	..	..	..	..	..	..	..	974	0·045	9·9
	1110±2	41	7·3	1110	41·5	7·2	1093	53	7·1	1174	36·9	7·3
	1404±2	39	7·8	1400	38·4	7·7	1387	29	7·8	1408	51·05	7·6

in a third group with end-point energy of 740 ± 4 keV. A subsequent subtraction analysis of the Fermi-Kurie (Fig. 1) plot has resulted in beta groups with energies 515 ± 5 and 405 ± 7 keV. The present results on the end point energies of intensities and log ft values of different beta groups are given in Table I along with the earlier results. Beta groups with end point energies below 200 keV could not be analysed due to the falling of the efficiency of the plastic detector in the spectrometer. However, its efficiency could be maintained unity down to 100 keV under special improved conditions.

The data on the beta groups and the results on a subsequent internal conversion measurements[5] are incorporated in a decay scheme as in (Fig. 2). This detailed and accurate beta spectrum analysis of ^{143}Ce has provided very accurate end point energies of the beta groups, their intensities and log ft values.

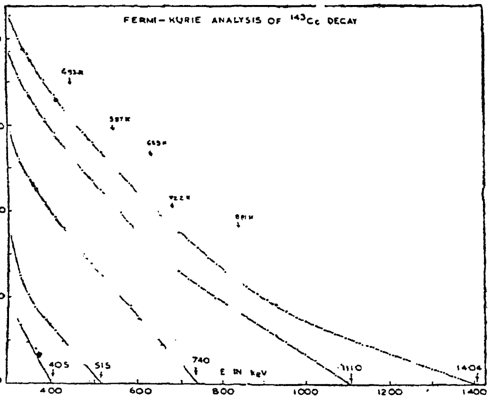

FIG. 1. Fermi-kurie analysis of the 33 hr. beta decay of ^{143}Ce shape corrected high energy beta groups are used for subtraction from the gross spectrum.

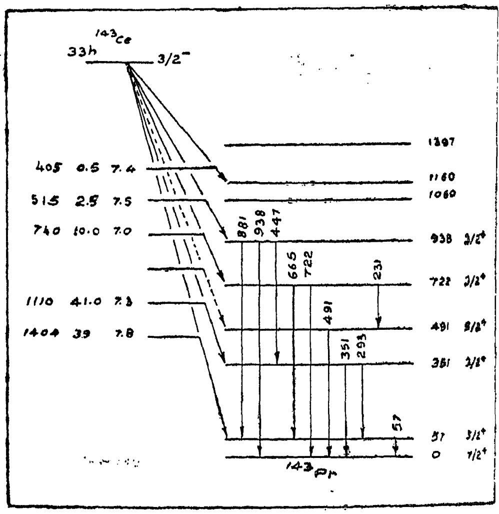

FIG. 2. Partial decay scheme of ^{143}Ce. Th beta energies, log ft values and spins of the indicat levels are incorporated from the present measur ments.

ACKNOWLEDGEMENT

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1. Gopinathan, K. P., Joshi, M. C. and Sarm E. A., *Phys. Rev.*, 1964, 136, 1312.
2. Megli, D. C., Potnis, V. R. and Mandevill C. E., *Nucl. Phys.*, 1968, 107 A, 117.
3. Sunyar, A. W. and Thieberger, P., *Bull. A Phys. Soc.*, 1966, 11, 407.
4. Nagarajan, T., Ravindranath, M. and Venka Reddy, K., *Nucl. Instr. Meth.*, 1969, 6 210.
5. Venkata Ramaniah, K., *Ph.D. Thesis*, Andh University, 1974 (unpublished).
6. Raju, M. L. N., Sastry, D. L. and Kondi E., *Nuovo Cimento*, 1968, 56 B, 29.
7. Venkata Ramaniah, K. and Venkata Redd K., *J. Phys.* (London), 1977, 3 (5); 667.

THE RESIDUAL PARAMAGNETISM IN COBALTIC COMPLEXES

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ABSTRACT

Temperature independent paramagnetism (χ_p) values of cobaltic complexes of the type [Coen$_2$ (R) Cl] Cl$_2$, where 'R' is an amine have been calculated on the basis of Griffith and Orgel's theory. The calculated (χ_p) values were compared with those experimentally obtained from magnetic susceptibility measurements. The orbital reduction factor "κ" values in all the cases were obtained to examine bonding effects. A decreasing order of the calculated (χ) values in terms of the amine 'R' is established.

INTRODUCTION

DEVELOPMENT in the ligand field theory lead interest in the magnetic studies of cobaltic complexes. On the basis of ligand field theory Griffith and Orgel[1] calculated the amount of the second order paramagnetism (χ_p) for the octahedral complexes of trivalent cobalt. They showed that in the cobaltic complexes, since the ground state of the system is $t_2{}^6\ {}^1A_{1g}$, only the high frequency term representing the temperature independent paramagnetism (Van Vleck paramagnetism) contributes to the paramagnetic susceptibility. In the present investigation the authors have made use of Griffith and Orgel's theory and calculated the χ_p values of the cobaltic complexes.

EXPERIMENTAL

Magnetic susceptibilities and the (χ_p) values of the cobaltic complexes of the type [Coen$_2$(R)Cl]Cl$_2$ where 'R' is an amine have been reported by the authors[2]. Absorption spectra of all these complexes in the visible region were recorded on the automatic Beckman DB-Spectrophotometer. It was possible to record directly a curve representing percentage transmittance against wavelength. The maximum absorption (λ_{max}) in these complexes was found to be in the region of wavelength 490 mμ and 540 mμ. By using the information obtained from these measurements (χ_p) values were calculated theoretically. Following Griffith and Orgels relation was used for this purpose[3].

$$\chi_p = \frac{2N\beta^2\langle[t_2{}^5e\ {}^1T_{1g}{}^z\,|L_z|\,t_2{}^6\ {}^1A_{1g}\rangle]^2}{K},\ i.e.,$$

$$\chi_p = \frac{4\cdot085}{K} \qquad ..\ (1)$$

where $T_1{}^z$ is the 'Z' component of T_1, 'β' is the Bohr magneton and K is expressed in cm^{-1}. K was obtained experimentally as $K = 1/\lambda_{max}$, values thus obtained alongwith experimentally observed values are reported in Table I.

DISCUSSION OF THE RESULTS

Cobaltic complexes are in general of low spin type, with $t_2{}^6e^0$ configuration. All the cobaltic complexes studied in the present investigation are diamagnetic[2]. They are therefore of low spin type with ($t_2{}^6\ {}^1A_{1g}$) as ground state and ($t_2{}^5\,e\ {}^1T_{1g}$) as excited state. It is observed from the results in Table I that the agreement in calculated and observed values of χ_p is excellent for the complexes formed with ortho-chloroaniline, meta-anisidine, meta-phenetidine and benzedine. For the other complexes the calculated values of χ_p are much higher than the observed ones. According to Griffith and Orgel the estimated probable errors in their theoretical calculations is about 20%. In the data for para-anisidine-complex and *m*-chloroaniline complexes, the disagreement is much greater. The lack of good agreement between the observed and calculated χ_p values may partly be due to the uncertainties involved in use of the susceptibility data of the chloride ion reported by different workers is divergent. Further, the diamagnetic susceptibilities of the amines when complexed with Co (III) may not be the same as that of free amines. In aromatic amines the values of the diamagnetic anisotropy in the free amine and complexed amine are likely to be different. This may be the case with complexes with ortho-phenetidine, pyridine, and *m*-toludine.

Orbital Reduction Factor :

It is significant to note that the observed values are always less than the theoretical estimates. The results probably reflect bonding effects which were not taken into consideration in Griffith and Orgel's theory. Their theory assumes that the 't_2' orbitals approximate very closely to the '*d*' orbitals. This would however, not be the case in the presence of bonding which has been neglected in the theory. By introducing a factor 'κ^2' on the r.h.s. of the expression (1) it is possible to make an allowance for the departure from pure '*d*' orbitals. This was done by Griffith[3]. He suggested a modified expression for the χ_p term.

$$\chi_p = 2N\beta^2 \cdot \frac{24\cdot\kappa^2}{K} \qquad ..\ (2)$$

where 'κ' is the orbital reduction factor. For pure '*d*' orbitals 'κ' is equal to 1 and low spin complexes 'κ' will be close to unity. Thus, (1−κ)

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TABLE I

Observed and calculated (χ_p) values of cobaltic complexes

Sl. No.	Formula of the complex (complexing ligand)	χ_p (obs.) in c.g.s. units	λ_{max} in mμ from graphs	Energy of transition in cm^{-1} $K=1/\lambda_{max}$	χ_p(calc.) $\chi_p=4\cdot085/K$ in c.g.s. units	Orbital reduction factor "k"	Extinction coefficient "E"
1.	$[Coen_2(C_2H_2N_4)Cl]Cl_2$ (dicyandiamide)	131·73	495	20190	202·2	0·8071	197·8
2.	$[Coen_2(C_6H_5N)Cl]Cl_2H_2O$ (pyridine)	153·24	510	19610	208·3	0·8576	223·9
3.	$[Coen_2(C_6H_4\cdot NH_2\cdot OC_2H_5)Cl]Cl_2$ (ortho-phenetidine)	153·70	510	19610	208·3	0·8590	406·5
4.	$[Coen_2(C_6H_4\cdot NH_2\cdot CH_3)Cl]Cl_2\cdot H_2O$ (meta-toluidine)	166·14	520	19230	212·4	0·8844	98·0
5.	$[Coen_2(C_6H_4\cdot NH_2\cdot Cl)Cl]Cl_2$ (meta-chloroaniline)	171·12	510	19610	208·3	0·9063	189·4
6.	$[Coen_2(C_6H_4NH_2\cdot OCH_3)Cl]Cl_2\cdot H_2O$ (para-anisidine)	186·40	510	19610	208·3	0·9459	249·4
7.	$[Coen_2(C_6H_4\cdot NH_2\cdot Cl)Cl]Cl_2$ (ortho-chloro-aniline)	208·39	515	19420	210·4	0·9953	227·8
8.	$[Coen_2(C_6H_4\cdot NH_2\cdot OCH_3)Cl]Cl_2$ (meta-anisidine)	208·53	520	19230	212·4	0·9909	116·6
9.	$[Coen_2(C_6H_4\cdot NH_2\cdot OC_2H_5)Cl]Cl_2$ (meta-phenetidine)	211·48	520	19230	212·4	0·9979	219·3
10.	$[Coen_2(C_6H_5\cdot NH_2\cdot C_6H_5NH_2)Cl]Cl_2$ (benzedine)	214·91	540	18520	220·1	0·9880	133·8

* $[Coen_2(C_6H_4NH_2\cdot CH_3)Cl]Cl_2\cdot H_2O \rightarrow \kappa = 0\cdot92$ (ortho-toluidine)

$[Coen_2(C_6H_4NH_2\cdot OCH_3)Cl]Cl_2\cdot H_2O \rightarrow \kappa = 0\cdot82$ (ortho-anisidine)

$[Coen_2(C_6H_4NH_2\cdot CH_3)Cl]Cl_2 \rightarrow \kappa = 0\cdot65$ (para-toluidine)

$[Coen_2(C_6H_4\cdot NH_2\cdot OC_2H_5)Cl]Cl_2 \rightarrow \kappa = 0\cdot74$ (para-phenetidine)

$[Coen_2(C_6H_5NH_2)Cl]Cl_2 \rightarrow k = 0\cdot51$. (Aniline).

is a measure of the deviation of actual orbitals from being pure '*d*' orbitals. From the study of a large number of cobaltic complexes, Kanekar and Nipankar[4], Assumssen and Ballhausen[5] and Datar and Patankar[6] calculated the values of orbital reduction factor 'κ' using the formula 'κ' $= \sqrt{\chi_p\,(\text{obs})/\chi_p\,(\text{calc})}$. The authors in the present investigation calculated 'κ' values in the same manner which are reported in Table I.

The values of 'κ' reported fall within the range of 0·8 to 1·0. Findings of the other authors[4,5,6] indicated that the values of 'κ' vary between 0·7 and 1·0 for ligands containing nitrogen and oxygen donors. The observations made by the authors in the present investigation are similar to the results of all these workers.

A careful scrutiny of 'κ' values shows that for the complexes with *o*-chloroaniline, *m*-anisidine, *m*-phenetidine and benzedine, the orbital reduction factor is unity whereas for the other complexes the magnitude varies between 0·8 and 1·0. It is therefore, apparent that the bonds formed between the metal atom and the ligands pyridine, *o*-phenetidine, *m*-toluidine, *m*-chloroaniline, *p*-anisidine and dicyandiamide are relatively stronger than those formed with the former ligands. Among the ligands forming a stronger bond, the one formed with dicyandiamide is the strongest and it is noteworthy that this is the only aliphatic amine used as the complexing ligand, the others being aromatic with the exception of pyridine. A comparison of 'κ' values for phenetidines and chloroanilines shows that the bonding effect in the case of meta-chloroaniline is much greater than that in ortho-chloroaniline whereas this order is exactly reverse in the case of ortho- and meta-phenetidines. The smaller bonding effect in the case of *o*-chloroaniline may be attributed to the steric factor. However, it is not clear why OC_2H_5 in *o*-phenetidine should not affect the 'κ' value in the same manner, since Cl and OC_2H_5 are known to be similar in their behaviour as regards their electro-negativity and size of the substituent. It is known that though Cl group is electron releasing, many times it acts as electron attracting group like NO_2 group. It is possible that in *o*-chloroaniline it withdraws electronic charge from the nitrogen atom of NH_2 group thus decreasing its power for bond formation. This may account for the weak bonding of this ligand as compared with that of *o*-phenetidine.

Comparative study of 'κ' values for isomeric complexes :

An attempt has been made to examine the orbital reduction factors for the isomeric substituted ligands in these complexes with a view to study the effect of substituents on the bonding. The authors[2] have measured the magnetic susceptibility values of a few complexes with substituents like CH_3, OCH_3, OC_2H_5 and Cl groups. For a complete comparison it is necessary to have the data on all position isomers. Datar and Patankar[6] have calculated the values of orbital reduction factor from the knowledge of the magnetic susceptibility of the cobaltic complexes of aliphatic and aromatic amines in this laboratory. His values have been taken for this comparison. The values taken from Datar and Patankar are reported at foot-note of Table I.

A glance at the values of 'κ' suggests that introduction of a substituent in aniline in various positions in benzene nucleus causes a decrease in the value of orbital reduction factor indicating that these substituents weaken the bond between metal and the ligand to a considerable extent. Among the isomeric compounds it is observed that in anisidines the lowering in 'κ' values is indicated by the order meta > para > ortho.

This order is slightly different for phenetidines, it being meta > ortho > para. In toluidines and chloro substituted anilines this order is found to be ortho > meta > para. It is therefore, concluded that in the former series, the strength of the bond is highest for meta compounds, the decrease in the trend of order being ortho > para > meta and para > ortho > meta respectively for anisidines and phenetidines. In the latter the same can be represented as para > meta > ortho. Since the substituents used in the present investigation are all ortho-para orienting, it is expected that for ortho-para isomeric compounds the electro-negativity of nitrogen in these compounds, should be greater than that in meta compounds. Accordingly, corresponding to higher electronegativity of nitrogen, one should expect a stronger bond between the metal and the ligand, and the order of the strength of the bond should be ortho > para > meta. This reasoning appears to be justified from 'κ' values for anisidines and is in partial agreement for phenetidines. In both these series the ortho and para compounds form the stronger bonds between the metal and nitrogen as compared to meta compounds. The slight difference in the order of 'κ' values between ortho and para compounds for anisidines and phenetidines may be attributed to the steric factor in the ease of *o*-phenetidine, the OC_2H_5 group being larger than OCH_3 group. The results of chloro and methyl substituted anilines are however, contrary to these expectations. It appears that in these compounds some other factor is more effective in non-release of electronic charge towards the nitrogen atom thus accounting for the weaker bonds in ortho substituted compounds.

Calculation of extinction coefficient

The extinction coefficient was calculated by using the expression based on Lambert Beer's law—

Log I/I_0 = ECL where log I/I_0 = optical density at λ_{max}.
C = Concentration of the solution
L = Length of the path which is 1 cm and
E = Extinction coefficient

Directly from the graph we get the percentage transmittance corresponding to λ_{max}. The percentage transmittance was converted into optical density by the formula O.D. = 2 − log T, where T = percentage transmittance at λ_{max}.

It has been stated earlier that the octahedral cobaltic complexes show an absorption band in the visible region which can be assigned to the transition ${}^1A_{1g}$-- ${}^1T_{1g}$. The band observed in the region of 490 to 540 mμ in these complexes has been ascribed to *d–d* transition ${}^1A_{1g} - {}^1T_{1g}$. This is also observed from the experimentally observed values of extinction coefficients reported in Table I which are of the order of 100 as expected for *d–d* transitions.

The data indicate that the χ_p (calculated values decrease in the following order of 'R'. Benzedine > meta-phenetidine ∼ meta-anisidine ∼ meta-toluidine > ortho-chloro-aniline > para-anisidine ∼ meta-chloroaniline > ortho-phenetidine > pyridine > dicyanidiamide.

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1. Griffith, J. S. and Orgel, L. E., *Trans. Faraday Soc.*, 1957, 53, 601.
2. Dodwad, S. S. and Datar, M. G., *Curr. Sci*, 1977, 46 (1), 7.
3. Griffith, *Theory of Transition Metal Ions*, Cambridge University Press, 1961, p. 284.
4. Kanekar, C. R. and Nipankar, S. V., *J. Ind. Chem. Soc.*, 1966, 43, 397.
5. Assmussen, and Ballhausen, *Acta. Chem. Scand.*, 1957, 11, 497.
6. Datar, M. G. and Patankar, V. P., *J. Univ. Bom.* (In press).

CHROMATOGRAPHIC SEPARATION OF PHOSPHITE AND PHOSPHATE IONS

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ABSTRACT

Separation of P (III) and P (V) is investigated. The influence of solvents and pH on the migration were studied. Their migrational behaviour is explained on the basis of solubilization and complexation. The degrees of separations were indicated by R_f values and the ions were quantitatively assayed photometrically.

INTRODUCTION

LITERATURE on chromatographic separation of P (III) and P (V) is sparse, most of the work in the past having been confined to polymeric phosphates[1-6]. The separation of phosphite and phosphate though unusual, assume importance in agriculture. Phosphorus is an essential ingredient of fertilizers, and plants absorb both phosphite and phosphate. While the soluble phosphate is beneficial, phosphite can considerably inhibit oxygen uptake and hamper nitrogen fixation by the roots in certain cases[7]. This would therefore necessitate detection and estimation of small quantities of phosphite in the presence of major quantities of phosphate in fertilizers. Paper chromatography is an ideal tool for this.

The principle adopted for the separation is based on the stereochemical differences of the phosphorus ions in the two states of oxidation. Phosphite and phosphate have both tetrahedral configurations by sp^3 hybridization[8,9]. However, while the tetrahedron of the phosphite is distorted, that of phosphate has a symmetrical distribution of valences. The distortion of a stereo-configuration usually imparts a greater covalent nature to phosphite and so it may be expected to complex with less polar solvents better than the phosphate, which is also borne out by our experiments; phosphite and phosphate migrate with R_f 1·0 in water. But phosphate slows down in less polar solvents like alcohols. However the gap in migration was not large enough to result in good separation but the ΔR_f could be enlarged by using mixed solvent systems. The present work makes use of the Rutter's technique[10] of circular paper chromatography and offers suitable explanations for the differential migrations of the two valences.

EXPERIMENTAL

Analytical grade NaH_2PO_3, Na_2HPO_3, NaH_2PO_4 and Na_2HPO_4 were dissolved in water to prepare 1·0 M solutions. The pH of these solutions was found to be 4·5, 8, 5 and 9 respectively. 1 μl. of each solution would contain 30·970 μg. of phosphorus. Phosphite and phosphate solutions were mixed in equal quantities as and when required. The solutions were spotted using an 'Agla' micrometer syringe (least count ± 0·2 μl). Chromatographic band of phosphite can be developed with 1% ferric chloride solution followed by 1% sulpho salicylic acid solution[1]. The technique is sensitive to 0·5 γ of phosphorus. Phosphate ion gives a blue coloured band when sprayed successively with 5% ammonium molybdate solution containing 35% nitric acid and 0·25% stannous chloride dissolved in 20% HCl[11]. The reagent is sensitive to 1·25 γ of phosphorus. The choice of eluents was guided by their polarity, viscosity and solubilization[12].

RESULTS AND DISCUSSION

In the case of *monobasic* salts of phosphite and phosphate the separation was poor as shown by the smallness of the ΔR_f. In highly polar solvents like water, and aqueous ammonia or methyl amine (25% W/V), both ions moved with equal facility to the solvent boundary, the phosphite migrated as a well defined band, while the phosphate showed some diffusion. With less polar solvents like butanol and others, the migration was negligible for both the ions. At any rate no satisfactory separation was possible with these eluents (Table I).

The results obtained with *dibasic* salts of phosphite and phosphate were also more or less the same as with the monobasic salts. However, we were able to satisfactorily separate the two ions using methanol-ammonia, methanol-methylamine, dioxane-ammonia, and dioxane-methylamine.

Certain interesting points are revealed by the above study. Though both the ions migrate with the same R_f in water, the phosphate showed diffusion while the phosphite migrated as a well-defined band. The same was the case with strongly ammoniated water as eluent. This difference in quality of migration may be attributed to the relative strengths of the two acids. The pK_2 of phosphoric acid is 7·21 and that of phosphorous acid is 6·15; in the second stage of dissociation H_3PO_3 is about six times stronger than water and about ten times stronger than H_3PO_4. We are of the opinion that the greater acidity goes to improve the quality of migration in the case of phosphite. The dibasic phosphate is likely to hydrolyse reversibly as $Na_2HPO_4 \rightleftharpoons NaH_2PO_4$, while Na_2HPO_3 does not. Since phosphate changes composition during migration its course is marked by diffusion. That Na_2HPO_4 undergoes reversible hydrolysis during migration was proved by spotting the chromatogram directly with NaH_2PO_4, when the same type of diffusion was observed, vide item 1 of Table I.

The situation was improved by partially replacing water with organic solvents, such as methanol or dioxane. With 50 : 50 dioxane-water, phosphate diffused with an R 0·86 while the phosphite migrated with an R_f 0·71. The diffusion band of phosphate overlapped that of the phosphite, making the separation ineffective. With 50 : 50 dioxane-ammonia as eluent the diffusion of phosphate was considerably retarded, whereas the migration of phosphite showed an R_f of 0·52. The differential migration in dioxane-ammonia eluent can be explained as follows : Na_2HPO_4 may form

TABLE I

Chromatographic behaviour of phosphite and phosphate mixture

No.	Eluent	Dielectric constant	R_f values of NaH_2PO_3	R_f values of NaH_2PO_4	R_f values of Na_2HPO_3	R_f values of Na_2HPO_4	$\triangle R_f$
1.	Water	78·54	1·0	1·0^d	1·0	1·0^d	
2.	Methanol	32·63	0·75	0·80^d	0·52^d	0·50^d	
3.	Ethanol	24·3	0·35^d	0·54^d	0·24^d	0·00^d	
4.	*n*-Propanol	20·1	0·00	0·45^d	0·00	0·00	
5.	*n*-Butanol	17·1	0·00	0·35^d	0·00	0·00	
6.	*n*-Amyl alcohol	13·9	0·00	0·25^d	0·00	0·00	
7.	Pyridine	12·3	0·00	0·62^d	0·00	0·00	
8.	Dioxane	2·21	0·00	0·00	0·00	0·00	
9.	Ammonia**		1·00	1·00^d	1·0	1·0^d	
10.	Methylamine$^\omega$		1·0	1·0^d	1·0	1·0^d	
11.	MeOH : HCl (99:1)		0·92	0·95			
12.	*n*-PrOH : HCl (99:1)		0·80	0·75^d			
13.	*n*-AmOH : HCl (99:1)		0·79^d	0·67^d			
14.	MeOH : Water (90:10)		0·78	0·80^d			
15.	EtOH : Water (90:10)		0·49	0·61^d			
16.	EtOH : *n*-BuOH : HCl (49:50:1)		0·80	0·87^d			
17.	*n*-PrOH : *n*-BuOH : HCl (49:50:1)		0·80	0·77^d			
18.	Dioxane : Water (50:50)				0·71	0·86^d	
19.	Dioxane : Ammonia (40:60)*				0·80	0·57^d	0·23
20.	Dioxane : Ammonia (50:50)*				0·52	0·29^d	0·23
21.	MeOH : Ammonia (90:10)*				0·48	0·26^d	0·22
22.	MeOH : Ammonia (70:30)*				0·61	0·42^d	0·19
23.	Dioxane : Methylamine (50:50)*				0·50	0·35^d	0·15
24.	MeOH : Methylamine (90:10)*				0·46	0·27^d	0·19
25.	MeOH : Methylamine (80:20)*				0·49	0·34^d	0·15

** 25% in water (W/V). *d*—Diffuse. * Good separation.

TABLE II

Quantitative estimation of phosphite and phosphate after separation

Quantity spotted: 92·91 μg each of phosphite and phosphate

No.	Eluent	Phosphite as P		Phosphate as P		$\frac{P(V)}{P(III)+P(V)} \times 100$ (%)**
		Estimated*	Error	Estimated*	Error	
		(μg)	(μg)	(μg)	(μg)	
1.	Dioxane: Ammonia † (50:50)	90·1	−2·81	93·5	+0·59	50·93
2.	Dioxane: Methylamine † (50:50)	90·6	−2·31	91·7	−1·21	50·30
3.	Methanol: Ammonia					
	(*a*) 90:10	94·2	+1·29	90·9	−2·01	49·11
	(*b*) 70:30	90·3	−2·61	95·1	+2·19	51·30
4.	Methanol: Methylamine					
	(*a*) 90:10	93·8	+0·89	90·5	−2·41	49·10
	(*b*) 80:20	89·9	−3·01	95·7	+2·79	51·56

* Average of four estimations. † 25% in water (W/V).

** Redox ratio: 50% = No reduction or oxidation. More or less than 50% indicates oxidation or reduction respectively.

with ammonia a microcosmic salt-like complex[13,14], with the result the diffusion of phosphate is retarded. Since the phosphite has no tendency to form a similar complex, it migrates with a different R_f value. Other eluents like methanol-ammonia, methanol-methylamine, dioxane-methylamine work very similar to that of dioxane-ammonia system. With higher percentages of ammonia or methylamine, the influence of water comes into play and phosphate diffusion catches up with the phosphite band. However, good separations were possible with methanol and dioxane in admixture with ammonia (25% aq) or methylamine (25% aq).

Estimation of phosphite and phosphate after separation

The positions of phosphite and phosphate ions on the chromatogram were located with the help of pilot experiments wherever good separations were achieved. The bands were then cut out, shredded and the ions were extracted with 1% HCl. The phosphate ions in the extract was estimated photometrically by the modified heteropoly molybdenum blue method described by Lueck and Boltz[15]. The phosphite in the extract was oxidised to phosphate by digesting with fuming nitric acid and the resulting phosphate was estimated photometrically as before. The results are presented in Table II.

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1. Ebel, J. P., *Mikrochim. Acta*, 1954, 6, 679; *Anal. Abstr.*, 1955, 2, 901.
2. Volmar, Y., Ebel, J. P. and Bassili Fawzi, Y. *Bull. Soc. Chim.*, France, 1953, 20, 1085 *Anal. Abstr.*, 1954, 1, 466.
3. Kuzmenko, M. V., Alekseeva, L. A. an Moizhes, I. B., *Trudy Leningr Nauchnoi sled. Proekt. Inst. Oxnov. Khim. Prom* 1971, 4, 244 ; *Anal. Abstr.*, 1972, 23, 382
4. Cvjeticanin, N. M. and Obrenovic, I. D., *Bu Inst. Nuclear Sci. 'Boris Kidrich* (Belgrade 1961, 11, 173 ; *Chem. Abstr.*, 1962, 56; 19
5. Hans Hettler, *J. Chromatog.*, 1958, 1, 389.
6. Ohashi, S., Nakamura, T. and Katabuchi, *Ibid.*, 1969, 43, 291.
7. Wm. A. Bulen, and Frear, D. S., *Arch. Bi chem. Biophys.*, 1957, 66, 502.
8. Wells, A. F., *Structural Inorganic Chemist* 3rd Edn., Oxford, 1962, p. 648.
9. Phillips, C. S. G. and Williams, R. J. *Inorganic Chemistry*, Vol. I, Oxford, 19 p. 256.
10. Rutter, L., *Nature*, 1948, 161, 435 ; *Anal* 1950, 75, 37.
11. Feigl, F., *Spot Tests in Inorganic Analy* Elsevier, 5th Edn., 1958, p. 333.
12. Paul, A. and Janardhan, P. B., *Bull. Ch Soc.*, Japan, 1967, 40 (5), 1131.
13. Partington, J. A., *A Text-Book of Inorg Chemistry*, Macmillan, 1937, p. 623.
14. Remy, H., *Treatise on Inorganic Chemis* Vol. I. Elsevier, 1956, p. 638.
15. Lueck, C. H. and Boltz, D. F., *Anal. Ch* 1956, 28, 1168.

CHEMICAL EXAMINATION AND UTILISATION OF *CORDIA DICHOTOMA* KERNEL

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ABSTRACT

The seed kernel of *Cordia dichotoma* contains mainly fats and proteins. The fatty oil is rich in unsaturated acids and the defatted kernel protein contains a number of essential amino acids.

The fatty oil combines with sulphur like tobacco seed oil to form a non-sticky mass known as 'factice' which has good electrical insulation properties, and this substance is still under investigation.

CORDIA DICHOTOMA FORST f., belonging to the family Boraginaceae, is a medium-sized tree. It is widely distributed in India, especially in the warmer regions. Its wood, which is fairly strong, is said to be more durable in contact with water and is used for boat construction, agricultural implements, etc.

The tree yields yellowish brown to pink coloured fruits during May–July, popularly called 'sebestan'. The fruit, when ripe, has a sweetish transparent mucilaginous pulp surrounding a central seed. The latter is a hard nut accounting for one-tenth of the weight of the whole fruit. On breaking, it yields a light-coloured kernel. Both the mucilage and the kernel are reported to have useful medicinal properties[1,2]. The chemistry of the mucilage has already been studied by Tiwari *et al.* and reported elsewhere[3].

The kernels contain 46·3% of a fatty oil and 31·5% of proteins. The fatty oil and the proteins are studied in detail, and the results are now reported in this paper.

EXPERIMENTAL

The kernel was separated from the shell after crushing the seed and its proximate analysis was carried out[4]. The results are presented in Table 1.

Isolation of the kernel oil

The crushed kernel was extracted in a soxhlet with petroleum ether (60–80° C) and a fatty oil (46·3% yield on the weight of the kernel and 3·4% on the weight of the whole seed) was obtained after removal of the solvent. The physico-chemical characteristics of the oil were determined according to standard methods[4] and the results are given in Table II.

Composition of the kernel oil

The oil was saponified with alcoholic potassium hydroxide and the liberated fatty acids, after removing the unsaponifiable matter, were separated into solid and liquid fatty acids by the Twitchell's lead salt method[4]. The iodine value of the solid and liquid fatty acid fractions was determined. The saturated and the unsaturated acids were found to be in the ratio of 1:3.

TABLE I
Proximate analysis of seed kernel

	Per cent
Moisture	8·50
Crude protein	31·49
Fat (oil)	46·30
Crude fibre	4·31
Non-fibre carbohydrates	5·32
Inorganic matter (by difference)	4·08
Ash	3·55

TABLE II
Physico-chemical characteristics of seed oil

Colour	Pale yellow
Odour	Almost odourless
Refractive index (at 26° C)	1·4680
Specific gravity (at 26° C)	0·9126
Iodine value (Hanus)	119·6
Saponification value	142·0
Acid value	1·36
Unsaponifiable matter	0·9

The solid (saturated) acid fraction and the liquid (unsaturated) acid fraction were subjected to reversed phase paper chromatography, descending and circular, using Whatmann No. 1 filter paper impregnated with 10%

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liquid paraffin in benzene. It may be mentioned that the circular method gave better separations. The developing solvent system (mobile phase) was 90% acetic acid saturated with liquid paraffin. A solution of the fatty acids in acetone was used for spotting. The developed chromatogram, after drying for about an hour at room temperature, was dipped in dilute copper acetate (10 ml. of saturated solution diluted to 250 ml.) solution for 45 min. and then washed with running water for 1 hour. The paper was again dipped in a 0·4% solution of sodium diethyl dithiocarbamate for 15 minutes when concentric dark-brown bands appeared. By carrying out co-chromatography using authentic samples, the following fatty acids were detected:—palmitic, stearic, arachidic and behanic in the solid (saturated) acid fraction, and oleic and linoleic along with two other unidentified acids in the liquid (unsaturated) acid fraction.

The methyl esters of solid and liquid acid fractions were analysed by gas-liquid chromatography using a Willy-Giede, Berlin Model GCHF 18·3 gas chromatograph, with a stainless steel column (2 m. long and 4 mm. in dia.) containing 10% silicone grease on celite at 205° C with a thermal conductivity detector. Hydrogen was used as the carrier gas at a flow rate of 130–140 ml/min. The peaks were identified by comparison of their retention times with those of the methyl esters of authentic fatty acids, run under identical conditions. The relative percentages of acids were calculated by estimating the area of individual peaks after triangulation using the formula, peak height × width at half height. Relative percentage composition of individual acids in the solid (saturated) acid fraction was: palmitic 62·3%, stearic 26·0%, arachidic 7·4%, behanic 3·8% and an unknown 0·5%. The relative percentage composition of the individual acids in the liquid (unsaturated) acid fraction was: oleic and linoleic 90·2%, an unknown acid 7·9% and other unidentified acids 1·9%. As the methyl esters of oleic and linoleic acids have more or less the same retention time even at different temperatures in the column used, they could not be identified separately.

The percentage composition of the fatty acids of the seed kernel oil is as follows: palmitic acid 15·58%, stearic acid 6·5%, arachidic acid 1·85%, behenic acid 0·95%, oleic and linoleic acids (combined) 67·65% and other unidentified acids 7·47%.

Amino acid composition of the seed protein

As no single solvent isolated the protein portion completely, it was isolated from the defatted kernel powder by successive extractions with water, sodium chloride solution (10%), sodium hydroxide solution (0·3%) and ethanol (80%) and was obtained in 5·96%, 1·35%, 1·75% and 0·53% yields respectively. Each extract was separately hydrolysed with 6 N HCl following the method of Gupta and Garg[5] and each hydrolysate was analysed for amino acids by descending paper chromatography (two dimensional) taking authentic compounds for reference. Whatmann No. 1 paper (46 × 57 cm) was used with the solvent system *n*-butanol-acetic acid-water (4:1:5 v/v upper layer) for one direction and phenol-ammonia-water (80:3:20 v/v) for the second direction. The spots were developed with ninhydrin (0·1% in acetone) spray, followed by heating in an air oven at 110° C for 5-10 min. The hydrolysates from each extract as well as the combined extracts were chromatographed separately under identical conditions. The Rf values were calculated for the authentic (reference) amino acids in both the directions and by comparison with the calculated Rf values for those of protein hydrolysates, the following seventeen amino acids were identified: cystine, aspartic acid, serine, glycine, glutamic acid, threonine, lysine, histidine, arginine, tyrosine, alanine, methionine, valine, phenyl alanine, leucine, isoleucine and proline. Of these amino acids two pairs, *viz.*, methionine and valine (Rf 0·49 one direction and 0·72 second direction) and leucine and isoleucine (Rf 0·64 one direction and 0·79 second direction) were difficult to be distinguished on account of their identical Rf values. It is worthy of note that the amino acid fraction contains a number of essential amino acids, particularly cystine and methionine. Containing a good percentage of fatty oil and proteins, the seed kernel can serve as a good cattle feed.

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1. *The Wealth of India (Raw Materials)*, Vol. I C.S.I.R., New Delhi, 1950, p. 346.
2. Chopra, R. N., Nayar, S. L. and Chopra, I. C *Glossary of Indian Medicinal Plants*, C.S.I.R New Delhi, 1956, p. 77.
3. Tiwari, R. D. and Purnima Dave, *The Pr* *Nat. Acad. of Sci. India* Sec. A, 1965, **35**, 437
4. *Methods of Analysis*, A.O.A.C. Association Agri. Chemists, Washington, D C., 19 p. 418, 423, 429 and 434.
5. Gupta, D. R. and Garg, S. R., *Ind. J. Ap* *Chem.*, 1967, **30**, 155.

GALVANIC INDUCTION OF CELL LYSIS AND ITS EFFECT ON PROTEIN CONTENT AND SUCCINIC DEHYDROGENASE, GLUTAMATE DEHYDROGENASE ACTIVITY LEVELS IN LIVER OF SHEEP

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ABSTRACT

The induction of cell lysis by voltage gradient and its effect on protein content, succinate dehydrogenase (SDH) and glutamate dehydrogenase (GDH) activity levels were studied in the liver of sheep. The cell lysis was found to occur when the liver was exposed to direct current. It is also observed that as the voltage gradient increased from 10 to 30 volts d.c/cm, the cell lysis also increased and the protein content, SDH and GDH activities showed a decline. The decline of SDH activity was higher when compared to that of GDH.

INTRODUCTION

WHEN gastrocnemius muscle and medulla oblongata which have elongated cells were subjected to a selected voltage gradient, earlier investigators[17,19] have found the electromigration of subcellular components which were separated into acidic and basic proteins. The preliminary studies[16,18] showed that enzyme activities were changed in cathodal and anodal halves in which acidic and basic protein environments predominate. The same work is extended to the liver tissue which is the key centre for most of the metabolic pathways.

MATERIALS AND METHODS

Sheep of similar age and body size group were decapitated and the liver was excised, cooled to 0° C and preserved in the deep freeze. The tissue was washed in Krebs–Henseleit Ringer salt solution (Krebs and Henseleit[15], 1932) and the peritonial membrane was carefully removed. The tissue was blotted gently between the folds of Whatmann No. 1 filter paper.

VOLTAGE GRADIENT AND PREPARATION OF AH, KH, C_1 AND C_2

The voltage gradient was applied to the tissue as suggested by Nandakumar and Swami[12] (1974) as follows.

The liver was washed in fresh cold Krebs–Henseleit bicarbonate Ringer solution and sliced into 4 approximately equal bits using stainless steel blade. One of the bits was taken in a clean small pyrex beaker cooled at 5° C and this served as the control. The other three bits were subjected to voltage gradients of 10, 20 and 30 volts per cm respectively, for 20 minutes. Temperature of about 5° C was maintained in the set up so that the residual metabolism is reduced to minimum (Swami and Krishnamoorthy[11], 1964). At the end of 20 minutes, the liver was quickly sliced transversely into two halves in the centre and these were designated as the anode half and the cathode half depending on the proximity of the tissue to the electrode with which they were associated. The control bit was also sliced in a similar manner and each half was called C_1 and C_2.

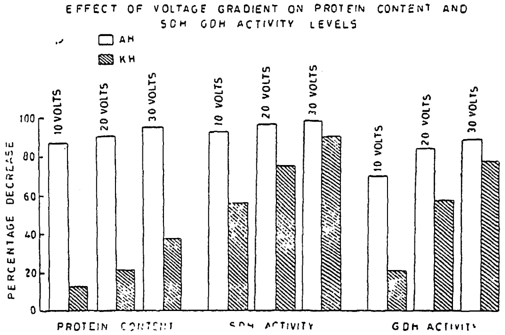

FIG. 1

The control and experimental tissue slices were homogenized in cold 0·25 M sucrose solution and also in distilled water separately with the help of YORCO tissue homogenizer at 2,500 rpm for two miuntes. 5% (W/V) homogenates were prepared at 0° C to 5° C and were subjected to centrifugation at 2,500 rpm for 15 minutes. The supernatant fractions were used for further assay. The protein content was determined by the method of Lowry *et al*[5] using bovine serum albumin as standard. The SDH (EC 1.3.99.1) activity was estimated by the method of Nachlas *et al*[4] (1960) as modified by Pramilamma *et al*[9] (1975) and GDH (EC 1.4.1.3) activity was estimated by the method of Lee and Lardy (1965) with a slight modifiction as described by Pramilamma and Swami[9] (1975) and the enzyme activity is expressed in μ moles of formazan/gm wet weight of tissue/hr.

RESULTS AND DISCUSSION

The application of voltage gradient induced a general decrease in protein content in both the AH and KH compared to the control. However in the experimental halves, the cathodal half showed minor decrement in the protein content while the AH showed a considerable decrement in protein content indicating that the destruction of the cellular protein was less in the KH as compared to AH as a consequence of application of voltage gradient. With the increase in voltage gradient from 10 to 20 and 30 volts/cm, the protein decrement or destruction was more in the anodal halves than in the cathodal halves.

This may be due to the fact that the tissue was exposed to the direct current, the galvanic field spreading from anodal (anelectronic field) to cathodal region (catelectronic field). When the tissue was exposed to such a field the cell lysis has occurred and it is also evident from the results that increase in the voltage gradient leads to the decrease in the protein content in both the AH and KH regions as compared to control.

Similarly, there is a decline in the activities of SDH, and GDH. The per cent inhibition in the case of SDH activity is mroe when compared to GDH activity. From this, we can infer that GDH is having a higher resistance than SDH to the applied voltage gradient. This may be due to the fact that the GDH is an ammonia detoxifying agent which is not as labile as SDH which is a parent enzyme in the oxidative metabolism.

From the above data it can be inferred that catelectronic field is relatively safer than the anelectronic field to induce cell-lysis. Such an application of selected voltage gradient may be helpful to arrest the tissue overgrowth which occurs in pathological conditions.

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1. Copenhaver, J. M. (Jr.), McShan, W. H. and Meyer, R. K., *J. Biol. Chem.*, 1950, 183, 73.
2. Dixon, M. and Webb, E. C., *Enzymes*, Academic Press, New York, 1958.
3. En Won Wu, C. O. and Muhro, H. W., Academic Press, New, York, 1969, p. 106.
4. Nachlas, M. M., Stanley, L. M. and Selligman, A. M., *J. Biol Chem.*, 1960, 253, 499.
5. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *Ibid.*, 1951, 193, 265.
6. Rao, K. V. K., *Ph.D. Dissertation* submitted to S.V. University, Tirupati, 1970.
7. Strecker, H. J., *Arch. Biochem. Biophys.*, 1953, 46, 128.
8. Dikshitulu, A. V. R., *Ph.D. Dissertation* submitted to S.V. University, Tirupati, 1975.
9. Pramilamma, Y., Kesava Rao, K. V. and Swami, K. S., *Ind. J. Exp. Biol.*, 1975, 13, 177.
10. Bergmeyer, H. A., Academic Press, Inc., New York, 1965.
11. Krishnamoorthy, K. V. and Swami, K. S., *J. Anim. Morphol. Physiol.*, 1964, 62, 58.
12. Nanda Kumar, N. V., *Ph.D. Dissertation* submitted to S.V. University, Tirupati, 1964.
13. Cavanaugh, G. M., *Formulae and Methods*, Vol. 4, Marine Biological Laboratory Wood Shole, Massachusetts.
14. Dikshitulu, A. V. R., Nanda Kumar, N. V. and Swami, K. S., *Annals of Allergy*, 1975, 34, 1.
15. Krebs, H. A. and Hanseleit, *J. Physiol. Chem.*, 1932, 210, 33.
16. Govindappa, S. and Swami, K. S., *Arch. Internat. Physiol. Biochem.*, 1966, 74, 259.
17. Muralikrishna Dass, P. and Swami, K. S., *Enzymologia*, 1972, 42, 3.
18. — and —, *Ind. J. Exp. Biol.*, 1968, 6, 19.
19. Bojji. Reddy, N. and Swami, K. S., *Ibid.*, 1971, 9, 32.

29TH INDIAN PHARMACEUTICAL CONGRESS, WALTAIR

The 29th Session of the Indian Pharmaceutical Congress will be held at the Andhra University Campus, Waltair, from 28th to 31st December, 1977.

Those who wish to present papers should contact the Convenor of the Scientific Services Committee Dr. V. P. Arya at D–312, Ciba–Geigy Research Centre, Goregaon East, Bombay 400 063, before 30th August, 1977.

LETTERS TO THE EDITOR

A COMMENT ON THE ANALYSIS OF VOLTERRA MODEL

THE purpose of this short note is to comment on the usual method of analysis of Volterra's model[1] for the population of several interacting species, with special reference to the review paper by Goel, Maitra and Montroll[2]. The n-species competing system is characterized by the system of equations

$$\frac{dN_i}{dt} = k_i N_i + \beta_i^{-1} \sum_{j=1}^{n} a_{ij} N_i N_j \qquad (1)$$

where N_i is the instantaneous population of the i-th species and k_i is its "rate constant". The interaction is represented by the second term on the right side of eq. (1) and corresponds to all possible binary encounters. The antisymmetric coefficients a_{ij} represent the strength of the interactions and the positive quantities β_i^{-1} are the "equivalence numbers".

In the work of Goel *et al.*[2], the "steady state" of the assembly was characterized by that set of populations $\{N_j\}$ for which

$$dN_j/dt = 0, \text{ for all } j. \qquad (2)$$

However we intend to point out here that such a set $\{N_j\}$ for which $dN_j/dt = 0$ for all j, is not a physical solution. It is clear from eqs. (1) and (2) that, not only the first order derivatives $\{dN_i/dt\}$ are zero, but all other finite order derivatives $\{d^m N_i/dt^m\}$ vanish simultaneously; for example from eq. (1)

$$\frac{d^2 N_i}{dt^2} = k_i \frac{dN_i}{dt} + \beta_i^{-1} \sum_{j=1}^{n} a_{ij} \times \left(N_i \frac{dN_j}{dt} + \frac{dN_i}{dt} N_j \right),$$

and each of the term on the right side is zero by eq. (2). Hence

$$\frac{d^2 N_i}{dt^2} = 0.$$

In like manner

$$\frac{d^m N_i}{dt^m} = 0 \qquad \text{(all } i \text{ and } m). \qquad (3)$$

Thus the set of populations $\{N_j\}$ will be totally flat as a function of time, as can be seen by a (Taylor) series expansion (also for earlier times by analytic continuation). But it is well known that the solution of the Volterra system shows oscillations in $\{N_j\}$[3] and at no finite time, $\{N_j\}$ is constant. Hence the equations(2) defining the "steady state" do not correspond to any physical solution. However we argue that all the relations derived in the work of Goel *et al*[2] are valid, provided we replace the "steady state" population by a long time average population $\{[[N_i]]\}$ defined by

$$[[N_i]] = \lim_{T\to\infty} \frac{1}{T} \int_0^T N_i(t)\, dt \qquad (4)$$

provided the limit exists. Then we can directly integrate eq. (1) from $t = 0$ to $t = T$, divide by T and take the limit $T \to \infty$, when we get

$$\lim_{T\to\infty} \beta_i \{T^{-1} \log N_i(T) - T^{-1} \log N_i(0)\}$$

$$= k_i \beta_i + \sum_{j=1}^{n} a_{ij} [[N_j]]. \qquad (5)$$

This was done in the work of Goel *et al.*[2]. However we wish to stress on an important difference. In the cited reference, the conservation condition [eq. (1.9) of ref. (2)] was utilized in the subsequent argument; but the conservation condition itself was derived from the "steady state" definition eq. (2). Our aim here is to derive the basic equation (1.6) of ref. (2) without invoking $dN_i/dt = 0$, for all i. After eq. (5) we argue as follows: $N_i(t)$ cannot be zero at any instant, since if $N_i(t_0) = 0$, then from eq. (1)

$$\frac{dN_i}{dt}(t_0) = 0, \qquad (6)$$

and following the same argument which led to eq. (3)

$$\frac{d^m N_i}{dt^m}(t_0) = 0 \qquad \text{(all } i, m). \qquad (7)$$

Thus the curve must be flat and continuing analytically to $t < t_0$, we see that $N_i(t) = 0$ at all time t (this will be so whenever the population of a species dies off with zero slope), which is an uninteresting situation. Furthermore, $N_i(t)$ cannot blow up, since we assumed, for the physically interesting case, that $\lim_{T\to\infty} 1/T \int_0^T N_i(t)\, dt$ exists. Thus $N_i(t)$ can neither vanish nor blow up. Hence the left side of eq. (5) vanishes and we have

$$k_i \beta_i + \sum_{j=1}^{n} a_{ij} [[N_i]] = 0, \qquad (8)$$

which is eq. (1.6) of ref. (2), provided we interpret $q_j = [[N_j]]$, as was suggested there. Once we derive eq. (1.6) of the cited reference, all the other relations derived therefrom stand valid.

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April 23, 1977.

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1. Volterra, V., *J. Conseil Permanent Intern. Exploration Mer III*, 1928, 1; translated in *Animal Ecology* by R. N. Chapman (McGraw-Hill, New York), 1931.
2. Goel, N. S., Maitra, S. C. and Montroll., E. W., *Rev. Mod. Phys.*, 1971, **43**, 231.
3. Rosen, R., *Dynamical System Theory in Biology* (Wiley-Interscience, New York), 1970.

RELAXATION TIMES AND ACTIVATION ENERGIES OF SOME SUBSTITUTED PHENOLS

RELAXATION times of four polar substituted phenols namely (A) 3, 5 dimethyl phenol, (B) 2, 3, 5 trimethyl phenol, (C) 2, 4, 6 trimethyl phenol and (D) 2, 4, 6 trichlorophenol have been measured in the dilute solution of benzene at 20, 25, 30 and 35° C at S-band. The free energies of activation for the process of dipole orientation and viscous flow have been calculated using Eyring's theory[1] of rate process. The results have been analysed in the light of molecular motions of the system and it is concluded that dipole orientation is contributed by both molecular as well as intramolecular rotation.

Dielectric studies of polar solutes in the non-polar solvent throw considerable light on various molecular and intramolecular forces. Magee and Walker[2,3] have investigated the relaxation times of some substituted phenols in a number of non-polar solvents. LeFerve[4] and Hiremath[5] have investigated the dielectric behaviour of phenols in the dilute solution of non-polar solvents and concluded that hydroxyl relaxation plays dominant role in the relaxation process of the phenols.

Due to their wide industrial and pharmaceutical applications the present study forms a significant contribution to the existing knowledge of the dielectric behaviour of some di and tri substituted phenols.

The dielectric constant (ϵ') and dielectric loss (ϵ'') have been measured on a S–*n* and microwave bench by a technique, due to Roberts and Von Hippel[7], later modified by Dekin and Works[8]. The relaxation time (τ) has been evaluated using Gopalakrishna fixed frequency method[9]. The compounds obtained from B.D.H., England, were of pure quality. The solvent (A.R.) was distilled before use.

The values of relaxation times and thermodynamic parameters are reported in Table I. I

TABLE I

Relaxation times and activation energies
($\lambda = 9·80$ cm)

Molecule	Temp. °K	$\tau \times 10^{12}$ sec	ΔF_ϵ Kcal/mol	ΔH_e Kcal/mol	ΔS_e Cal/mol	ΔF_η Kcal/mol	ΔH_η Kcal/mol	ΔS_η Cal/mol
3, 5 Dimethyl phenol	293	21·2	2·83	1·90	−3·17	2·90	2·52	−1·29
	298	20·1	2·86	1·90	−3·22	2·91	2·52	−1·30
	308	17·4	2·89	1·90	−3·21	2·93	2·52	−1·33
2, 4, 6 trichlorophenol	298	21·1	2·88	1·41	−4·93	2·90	2·52	−1·29
	303	19·8	2·91	1·41	−4·95	2·91	2·52	−1·30
	308	19·0	2·94	1·41	−4·96	2·93	2·52	−1·33
2, 4, 6 trimethylphenol	293	11·1	2·4	1·09	−4·64	2·90	2·52	−1·29
	298	10·6	2·5	1·09	−4·75	2·91	2·52	−1·30
	308	10·0	2·6	1·09	−4·74	2·92	2·52	−1·30
2, 3, 5 trimethylphenol	293	18·0	2·73	1·63	−3·75	2·90	2·52	−1·29
	298	16·9	2·75	1·63	−3·75	2·91	2·52	−1·30
	303	15·9	2·77	1·63	−3·76	2·92	2·52	−1·32
	308	15·3	2·88	1·63	−3·83	2·93	2·52	−1·33

is found that the relaxation times of all the molecules investigated here are found to decrease with increase in temperature. This is due to the decrease in the viscosity of the solvent as the temperature is raised. It is found from Table I that the relaxation time of 2, 4, 6 trichlorophenol is highest and the relaxation time of 2, 4, 6 trimethyl phenol is lowest in the investigated series whereas latter has largest molecular size. It is likely that in the case of 2, 4, 6 trichlorophenol the highly electronegative chloro group forms hydrogen bonding with the –OH group which in turn may hinder the intramolecular rotation of –OH group and increase the relaxation time. Sahni *et al.*[10] have interpreted the high value of relaxation times of many organic molecules as due to intermolecular hydrogen bonding.

The relaxation time of 2, 3, 5 trimethyl phenol is greater than 2, 4, 6 trimethyl phenol though both the molecules have almost similar molecular size. Also the relaxation time of 3, 5 dimethyl phenol is larger than the relaxation time of 2, 3, 5 trimethyl phenol and 2, 4, 6 trimethyl phenol though the former molecule is smaller than both the latter molecules in molecular size. This indicates that the molecule having irregular arrangement of the substituted groups experience greater hindrance in dipolar rotation.

The free energies of activation for the process of dielectric relaxation and viscous flow increase with temperature. Similar results have been observed earlier[11]. The entropies of activation are found to be negative indicating that activated state is more ordered than the normal one. Further the heats of activation and energies of activation for viscous flow are greater than those for dipole relaxation, since the former involves both rotation and translation, while the latter only rotation.

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Lucknow, *April* 14, 1977.

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---

1. Eyring, H., Glasstone, S. and Laider, K. J.; *The Theory of the Rate Process*, McGraw-Hill Co., New York, 1941, p. 548.
2. Magee, M. D. and Walker, S., *J. Chem. Phys.*, 1971, 55, 3068.
3. — and —, *Canadian, J. Chem.*, 1972, **50**, 2799.
4. LeFerve. R. J. W. *et al.*, *Aust. J. Chem.*, 1967, **20**, 623.
5. Hiremath, G. C. *et al.*, *Bull. Nat. Inst. Sci.*, India, 1965, 30, 113.
6. Gupta, K. C. and Pradhan, B. P., *Proc. Nat. Inst. Sci.*, India, 1959, 25 (A), 184.
7. Roberts, S. and Von Hippel, A., *J. Appl. Phys.*, 1946, 17, 610.
8. Dakin, T. W. and Works, C. N., *Ibid.*, 1947, 18, 610.
9. Gopalakrishna, K. V., *Trans. Faraday Soc.*, 1957, 53, 767.
10. Sahni, N. *et al.*, *Bull. Chem. Soc.*, Japan, 1976, 41, 973.
11. Dhar, R. L., Shukla, J. P. and Saxena, M. C., *Ind. J. Pure and Appl. Phys.*, 1972, **10**, 530.

KINETICS OF OXIDATION OF DIMETHYL SULPHOXIDE BY CHLORAMINE-T

The reaction DMSO–Mn^{3+} has been employed a a redox initiator for vinyl polymerisation[1]. We have recently reported a kinetic study of DMSO oxidation by Cr (VI) and Ce (IV)[2]. We report in this communication kinetics of oxidation of DMSO by Chloramine-T (CAT) in acid medium and OsO$_4$-catalysed oxidation of DMSO by alkaline CAT.

Oxidation of DMSO by CAT in acid medium

The reactions are first order in [DMSO], [CAT] and independent of [HClO$_4$] in the range studied. First order rate constants (k_1 min^{-1}) at varying [DMSO] are reported in Table I. The enhancement of the values of k_1 is due to the increase in the concentration of the DMSO.

Table I

Dependence on [DMSO] Temp.: 35° C

CAT = $5\cdot0 \times 10^{-4}$ M, HClO$_4$ = 0·05 M,
NaClO$_4$ = 0·025 M.

Aqueous medium		20% aqueous HOAc	
10^3 [DMSO] M	$10^3 k_1$ min^{-1}	10^3 [DMSO] M	$10^3 k_1$ min^{-1}
2·1	14·8	3·0	11·7
4·3	31·2	4·3	18·7
6·6	42·2	7·1	23·8
11·0	69·1	11·7	37·0

Variation of acidity (with respect to HClO$_4$) in the range 0·01 to 0·1 M in aqueous medium and in 20% aqueous HOAc leads to the same value of k_1 proving insensitivity to acidity. Second order rate constants (l·m^{-1} min^{-1}) in aqueous medium and in 20% aqueous HOAc are 7·3 and 4·0 respectively. This is at variance to the acidity dependence in the Cr(VI) and Ce(IV) oxidation of DMSO[2], where there is definite acid dependence. Ionic strength has been varied (NaClO$_4$, 0·025 to 0·1 M) and the effect is marginal. Added *p*-toluene sulphonamide has no effect on the rate. This has also been observed by Mushran and co-workers[4].

In view of the fact that several species of CAT (depending on pH) are current in literature, a systematic pH profile was attempted for the DMSO–CAT reaction. The reaction exhibits a rate maximum at pH 4·5. Second order rate constants at pH 4·5, 5·4 and 6·8 are 164·0, 59·0 and 1·4 respectively at 35° C. Such pH dependence has been observed earlier in the oxidation of alcohols and chlorination of amines by CAT, and is attributed to HOCl being the active species, whereas below this pH, RNHCl is postulated to be the active species which might have been responsible for the insensitivity to $HClO_4$ in the range 0·01 to 0·1 M. We rule out the mediation of dichloramine-T, because no second order dependence on [CAT] is obseved under these conditions. The reactions are greatly accelerated in aqeuous HOAc/NaOAc buffer (10% aqueous HOAc, NaOAc $= 0{\cdot}02$ M $k_2 = 16{\cdot}2$ $1.m^{-1}$ min^{-1} at 35° C). Such specific acetate ion catalysis has been observed earlier in DMSO-Br_2 oxidation[3]. The reactions are retarded in aqueous HOAc and aqueous ethanol at constant acidity, quite in consonance with the dipole-dipole nature of these reactions (Table II).

The reactions are sluggish from pH 6–8 and no perceptible oxidation occurs under alkaline conditions. To sum up it can be said that though the reaction has no dependence on [$HClO_4$] in the range of this study, it is not independent of [H^+] in the wider sense and the nature of the CAT species should be taken into account.

TABLE II

Solvent effect: Temp.: 35° C

DMSO = 0·0043 M, $HClO_4$ = 0·05 M,

$NaClO_4$ = 0·025 M, CAT = $5{\cdot}0 \times 10^{-4}$ M

Solvent	k_2 $1.m^{-1}$ min^{-1}	Solvent	k_2 $1.m^{-1}$ min^{-1}
10% HOAc	6·4	10% EtOH	5·2
20% HOAc	4·3	20% EtOH	3·7
30% HOAc	3·0	30% EtOH	2·5

It is already mentioned that the reaction of DMSO CAT in alkaline medium is sluggish and this has been catalysed by OsO_4. The following kinetic features are observed:

(i) Zero order in [DMSO] and [OH^-]. In the [DMSO] range $35{\cdot}0 \times 10^{-2}$ to $0{\cdot}44 \times 10^{-2}$ M, k_1 is essentially constant ($6{\cdot}8 \times 10^{-3}$ min^{-1}). In the [OH^-] range 0·1 to 0·4 M NaOH, $k_1 = 3{\cdot}0 \times 10^{-3}$ min^{-1} at 60° C.

(ii) *First order in [CAT] and [OsO_4]*

The dependence on [OH^-] in the oxidation of HCHO and CH_3CHO, is obvious from a consideration of the formation of aldehyde hydrate which loses a H. Such a possibility is not there in these reactions and these are independent of OH^- in the range studied.

TABLE III

NaOH = 0·1 M, CAT = $5{\cdot}0 \times 10^{-4}$ M, Temp.: 60 C

DMSO = 35×10^{-2} M

[OsO_4] 10^6 M	10^3 k_1 min^{-1}
3·9	1·6
7·8	2·8
11·7	3·7
15·6	5·8
19·5	6·8

Addition of *p*-toluene sulphonamide (0·0005 M) causes retardation which points to a specific negative salt effect. The following scheme is consistent with our kinetic data:

$$CAT + H_2O \rightleftharpoons RNHCl\,(CAT') + NaOH$$

$$CAT' + OsO_4 \xrightarrow{\text{Slow}} \text{Complex.}$$

$$DMSO + \text{Complex} \xrightarrow{\text{Fast}} \text{Dimethyl sulphone.}$$

$$R = CH_3 \cdot C_6H_4 \cdot SO_2$$

That, these reactions are dependent on a comple between CAT and OsO_4 is well proven by earli workers with other substrates[4]. The stoichiometr is 1:1 and the product is dimethyl sulphone.

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1. Devi, N. Ganga and Mahadevan, V., *Chemic Communications*, 1970, **13**, 797.
2. Radhakrishnamurti, P. S. and Padhi, S. C. *Curr. Sci.*, 1974, **43**, 715 and referenc cited therein.
3. Gibson, A. and Cox, B. G., *J. Chem. Soc.*, *Perk.* (II), 1973, **10**, 1355.
4. Sanehi, R., Agrawal, M. C. and Mushran, S. P. *Indian J. Chem.*, 1974, **12**, 311.

MICRO ANALYSIS OF Ni(II), Co(II), Pd(II) AND Ti(IV), Y(III), Zr(IV) BY COMBINED THIN-LAYER CHROMATOGRAPHY AND RING COLORIMETRY

THE combination of thin-layer chromatograph (TLC) and Weisz Ring Oven has been shown to be successful by Johri *et al.*[1-5] for the micro analysis of metal ions. The present paper relate to the use of some new chromogenic reagents lik diphenyl thiovioluric acid[6] (DPHTVA) tropolone and quercetin in the micro analysis of Ni(II), Co(II), Pd(II) and Ti(IV), Y(III), Zr(IV). Also, the use of fluorescent support on thin-layers has been avoided which creates difficulties with certain solvent systems.

Experimental

Analytical grade salts were dissolved in double distilled water to give 1 mg/ml of stock solutions.

Visualising Agents.—M/100 diphenyl thiovioluric acid (DPHTVA) in acetone, M/100 quercetin (Fluka) in alcohol, M/50 tropolone (Aldrich) and 0·1% solution of alizarin in alcohol were employed.

Separation of Ni(II), Co(II) and Pd(II)

1–3 μl of each of the test solutions was applied separately at three points on silica gel 'G' coated glass plate along the base line, from one end as a reference with help of Hamilton syringe. The mixture of the three ions was applied at the fourth point. The plate was developed by the ascending technique in MIBK–HCl (25 : 4 ; v/v) solvent system for 35 minutes so as to achieve clean separation of the ions. The plate was removed from the jar, thoroughly dried in a stream of hot air and the separated metal ions were located with sprays of diphenyl thiovioluric acid followed by 0·1% aq. NaOH.

Separation of Ti(IV), Y(III) and Zr(IV)

This separation was carried out on cellulose support using the solvent system, acetone-1 N HCl (25 : 2 ; v/v). The plate was developed for 15 minutes. The remaining procedure is the same as given in the previous separation.

Results are given in Table I.

Ring Colorimetric Determination of the Chromatographed Constituents

For the determination of the metal ions, one drop of the known mixture of Ni(II), Co(II) and Pd(II) at one end and a drop of the unknown test sample at the adjacent end of the thin-layer plate were applied. The plate was developed as described earlier. Half portion of the plate, containing the known separated constituents, was subjected to sprays of chromogenic reagent (Table I) leaving the other half unaffected. Next, the respective spot region of the particular metal ion on the thin layer (unsprayed) as judged by comparing it with the visualized portion of the plate was sucked out by means of a micro vacuum collector. The individual metal ions from the sucked out material were eluted with water on a Whatman No. 41 filter paper of 55 mm diameter, placed on the ring oven, maintained at 110° followed by its washings into the ring zone. The same procedure was repeated for the other metal ions. The individual metal ions were then treated with the reagents listed in Table II.

For the evaluation, the intensities of the coloured rings obtained with the different cationic species were compared visually with those of standard rings prepared separately[7] and corresponding to 1–10 μl of standard solution. Results of a few representative determinations are given in Table II.

Similar determination procedure was adopted for Ti(IV) Y(III) and Zr(IV).

In the case of Ni(II), Ti(IV) and Y(III), because of the yellow colour of the developed rings, the efficient visual comparison for their evalution was not possible. This difficulty, however,

TABLE I

Detection of ions after TLC separation

Metal ion	Spray reagent	Colour	R_f	Limit of Identification μg
Ni(II)	DPHTVA	Yellow	0·82	0·30
Co(II)	DPHTVA	Reddish-brown	0·64	0·09
Pd(II)	DPHTVA	Dark-brown	0·28	0·15
Ti(IV)	Quercetin	Brownish-red	0·62	0·20
Y(III)	Quercetin	Yellow	0·34	0·16
Zr(IV)	Quercetin	Yellow	zero	0·30

TABLE II

Representative results of determination

Ion determined	Developing agent	Colour of the ring	Ion taken mg/ml	Ion found mg/ml	Error %
Ni(II)	DPHTVA	Yellow	0·80	0·82	+2·5
			1·20	1·14	−5·0
			1·60	1·55	−3·1
Co(II)	DPHTVA	Reddish-brown	0·60	0·58	−3·3
			0·90	0·92	+2·2
			1·20	1·15	−4·1
Pd(II)	DPHTVA	Brown	0·75	0·75	0·0
			1·00	1·05	+5·0
			1·50	1·42	−5·3
Y(III)	Quercetin	Yellow	0·84	0·82	−2·4
			0·42	0·40	−4·7
			1·26	1·18	−6·3
Ti(IV)	Tropolone and 6 M HCl	Yellow	0·95	0·92	−3·1
			0·60	0·58	−3·3
			0·90	0·85	−5·5
Zr(IV)	1M HCl and alizarin i	Reddish-violet	0·75	0·72	−4·0
			1·00	0·94	−6·0
			1·50	1·60	+6·6

was over come by viewing the rings through blue glass both for standard scale as well as for test samples.

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1. Johri, K. N. and Mehra, H. C., *Micro Chem. J.*, 1970, 17, 642.
2. — and —, *Micro Chim. Acta*, 1970, p. 807.
3. —, — and Kaushik, N. K., *Chromatographia*, 1970, 3, 347.
4. — and —, *Sepn. Sci.*, 1971, 6, 741.
5. — and —, *Chromatographia*, 1971, 4, 351.
6. Dass, I. N. and Dutta, S., *Proc. Ind. Acad. Sci.*, 1938, 8, 145.
7. Weisz, H., *Micro Analysis by the Ring Oven Technique*, Pergamon, London, 1970.

DETERMINATION OF 3-AMINOPYRIDINE IN ETHYL METHYL KETONE MEDIUM

PETERSON *et al.*[1] carried out the potentiometric titrations of heterocyclic nitrogen bases such as pyridine with stannic chloride and other non-protonic acids in selenium oxychloride medium. They employed the technique of Muller "retarded" electrode system in the titrations. Garber *et al.*[2] effected the visual titrations of pyridine with stannic chloride in thionyl chloride medium using crystal violet and malachi green in solid form. In the present work an attemp has been made to titrate pyridine bases against stann chloride in ethyl methyl ketone medium. The en points were located with the aid of visual and pote tiometric techniques.

Ethyl methyl ketone (B.D.H.) was dried over a hydrous potassium carbonate for 24 hours and th fractionally distilled. 3-Aminopyridine (Koch-ligh and stannic chloride were used without purificatio Solutions of 3-aminopyridine and stannic chlori were prepared in ethyl methyl ketone. The ami solution containing 2 to 3 drops of methyl orange inc cator (0·1% solution in ethyl methyl ketone) was titr ted with stannic chloride. The colour changed fro yellow to pink at the end point.

Potentiometric titrations were performed w Elico pH meter employing glass electrode. Char teristic 'S'-shaped curves were obtained by plotti EMF against the volume of stannic chloride add The exact end point was located by the calculati method[3]. The results of visual and potentiome titrations are presented in Table I.

The results (Table I) show that 3-aminopyrid can be determined with an error of less than by visual titrations employing methyl ora as indicator. The determination of base can effected potentiometrically also with an error, than 1%.

TABLE I

	3-aminopyridine		
	Taken, molar	Found, molar	Error (%)
Visual	0·1000	0·0992	−0·8
	0·0750	0·0757	+0·9
	0·0500	0·0505	+1·0
Potentiometric	0·0750	0·0752	+0·3
	0·0500	0·0502	+0·4
	0·0250	0·0248	−0·8

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---

1. Peterson, W. S., Heimerzheim, C. J. and Smith, G. B. L., *J. Am. Chem. Soc.*, 1943, 65, 2403.
2. Garber, E. B., Peace, L. E. D. and Lucler, W. F., *Anal. Chem.*, 1953, **25**, 581.
3. Liteanu, C. and Mioscu, M., *Rev. Roum. Chim.*, 1968, **13**, 1185.

HABIT MODIFICATION DUE TO CAESIUM IONS DURING THE ELECTRODEPOSITION OF COPPER ON A COPPER (111) FACE

THE morphology of copper electrodeposit on the (111) plane of copper deposited from highly purified solution of acidified copper sulphate containing known concentrations of caesium sulphate was studied. Pyramids observed on (111) plane of copper when deposited from pure solution changed to layers, ridges and polycrystalline type of growth as concentration of caesium sulphate increased.

It is seen from the literature[1, 2] that there are very few reports on the effect of cations on the morphological and kinetic aspects during the prolonged deposition. Arsenic and antimony cause brittle and rough deposits when added to acid copper sulphate bath. The present experimental work was carried out to study the habit modification of copper electrodeposit, deposited on (111) plane of copper from acid copper sulphate bath in presence of caesium sulphate.

The cathode was (111) face of copper single crystal and the anode, a polycrystalline A.R. copper foil of area about 40 times larger than that of the cathode. The surface of the cathode was coated with araldite except the (111) plane of the crystal. This plane was mechanically polished on 4/0 emery paper using ethyl alcohol as a lubricant and electropolished in 50% of orthophosphoric acid at a constant cell potential of 1·2 volts in hydrogen atmosphere[3]. The electropolished surface of copper (111) face was washed with 10% orthophosphoric acid and finally with conductivity water and quickly transferred to the electrolytic cell. The electrolytic bath was a solution of 0·25 M pure copper sulphate and 0·1 M sulphuric acid[4]. A known concentration of caesium sulphate solution was added to the electrolytic bath and deposition was carried out at 10 mA/cm^2 to a thickness of 10 Coulombs/cm^2 at 25 ± 1° C. For each experiment fresh solution was used. The over-potentials were measured with respect to a freshly prepared copper electrode acting as a reference electrode, with the help of vacuum tube voltmeter (± 2 mv). The surface morphology was examined under a metallurgical microscope, and photomicrographs were taken.

A pyramidal type of growth was obtained when copper was deposited from purified copper sulphate at 10 mA/cm^2, as noticed by earlier workers[5, 6] (Fig. 1). It was observed that the height of the triangular pyramids decreased as the concentration of caesium sulphate was increased from 10^{-10} to 10^{-4} m/l. When the concentration of caesium sulphate was 10^{-7} m/l, the pyramidal type of growth completely changed to layer type of growth (Fig. 2). The layer type of deposit was completely transferred in to ridge type of growth at 10^{-5} m/l of caesium sulphate as illustrated in Fig. 3. The overpotential value decreased on the (111) plane as observed by earlier workers. The trend of decreasing and attaining a steady state remained but the initial as well as final values of overpotentials were always lower than in pure solution at the corresponding current density.

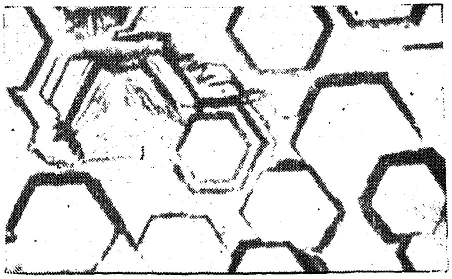

FIG. 1. Hexagonal pyramids and occasional triangular pyramids of copper deposited on copper (111) plane from pure copper sulphate bath at 10 mA/cm^2, × 625.

The above results indicate the remarkable effect of caesium ions on the habit modification of copper electrodeposit and on the overpotential during deposition. The mechanism of habit modification due to the caesium ions is under detailed study.

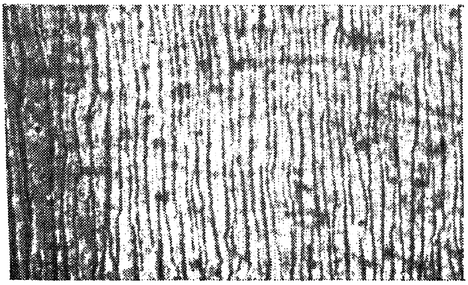

FIG. 2. Layer type of growth aligned in (110) direction when copper is deposited from acid copper sulphate bath in presence of 10^{-7} m/1 of caesium sulphate at 10 mA/cm^2, × 625.

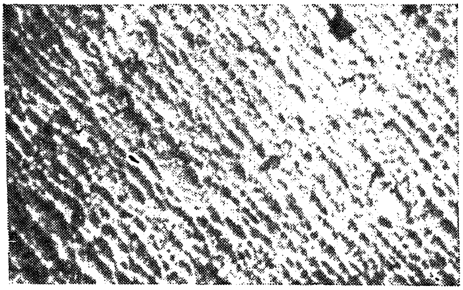

FIG. 3. Ridge type of growth on copper (111) plane from acid copper sulphate bath at 10 mA/cm^2 in presence of 10^{-5} m/1 of caesium sulphate, × 625.

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---

1. Barnes, S. C., *Trans. Electrochem. Soc.*, 1964, 113, 296.
2. *Metal Finishing, Guide Book Directory for 1970*, 38th annual edition, Metals and Plastics Publications, Inc., N.J., U.S.A.. p. 272.
3. Jacquet, P., *Compt. rend.*, 1933; 202, 402.
4. Nageswar, S. and Setty, T. H. V., *Proc. Ind. Acad. Sci.*, 1968, 68 A (4), 178.
5. Damjanovic, A., Setty, T. H. V. and Bockris, J. O. M., *J. Electrochem. Soc.*, 1966, 113, 129.
6. Nageswar, S., *Electrodeposition Surface Treat.*, 1975, 3, 195.

EFFECT OF SIMULATED HIGH ALTITUDE ON RED CELL MEMBRANE PERMEABILITY AS REVEALED BY INFLUX OF ^{86}Rb AND ^{22}Na

POTASSIUM homeostasis in acute hypoxia is controversial. Decreased plasma potassium (K^+) has been reported in hypoxic animals[1,2], while in human beings under similar conditions, concentration of K^+ in plasma either increases[3] or remains unchanged[4]. In the present investigation, trans-erythrocytic availability of sodium (^{22}Na) and potassium [using rubidium-86 (^{86}Rb) as a marker] in hypoxic rats have been characterised and the results have been correlated with red cell membrane Adenosine triphosphatase (ATPase) activity.

Materials and Methods

Adult male Sprague–Dawley rats, 4 months old, were fed with Hindlever Rat and Mice Feed and exposed to simulated altitude of 6100 m in a standard decompression chamber[5] at 26° C for 4 hours. Red Blood cell counts were made by standard haematological procedures. Influx of ^{86}Rb and ^{22}Na in erythrocytes *in vitro* was studied as per literature[6], using Nuclear Chicago Autogamma Spectrometer Model 4219. Potassium in plasma[7] and ATPase activity (basal and sodium potassium activated)[8] in red cell membranes were estimated colorimetrically. Protein was estimated by biret reaction[9].

Results and Discussion

Acute hypoxic condition was indicated by significant increase ($p < 0 \cdot 001$) in red cell counts of experimental rats (Table I). Results of Table I also reveal increase in the concentration of potassium in plasma ($p. < 0 \cdot 001$) an observation which is not in agreement with the previous findings[1,2]. Probably the drainage of intracellular potassium due to diminished oxygen supply[10-12] was responsible for greater availability of potassium in plasma. In the present series of experiments, influx of ^{22}Na in erthrocytes of experimental rats was the same as in control, while hypoxia led to 36% increase in influx of ^{86}Rb in red blood cells *in vitro*. This might be due to loss of red cell potassium *in vivo*. Both basal and sodium potassium activated ATPase activity in red cell membranes have increased ($p < 0 \cdot 01$) in hypoxic rats (Table I) which is not in agreement with the

TABLE I

Plasma potassium and ATPase activity in red cell stroma of hypoxic rats
(Data represent mean ± SEM. Figures in parentheses denote number of rats)

Group	RBC counts million/cmm	Potassium meq/litre	ATPase-pi literated in μg/hr/mg protein at 40°C	
			Basal	Na^+ K^+ activated
Control	5·6±0·2 (10)	6·4±0·1 (10)	1·7±0·16 (10)	2·1±0·2 (10)
Experimental	7·4±0·1 (10) $p < 0{\cdot}001$	8·6±0·1 (10) $p < 0{\cdot}001$	3·1±0·3 (6) $p < 0{\cdot}01$	3·5±0·2 (6) $p < 0{\cdot}01$

published report[13]. The observed increase in red cell membrane ATPase activity is probably responsible at least in part for the enhanced influx of ^{86}Rb in erythrocytes of acute hypoxic rats.

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1. Alfred, J., Pratt, D. C., Smith, S. F. and Ferguson, F. P., *Endocrinology*, 1955; 57. 450.
2. Ferguson, F. P., Smith, D. C. and Barry, J. Q., *Ibid.*, 1957, 60, 761.
3. Jezek, V., Onrednik, A., Daun, S. and Kronzkes, L., *Acta. Med. Scand.*, 1965, 177, 175.
4. Janoski, A. H., Whitten, B. K., Shields, J. L. and Hannon, J. P., *Fed. Proc.*, 1969, 29, 1185.
5. Sarkar, S. R., Singh, L. R., Banerji, R. and Chaudhuri, B. N., *Ind. J. Exp. Biol.*, 1977, 15, 142.
6. Dunn, A. and Arditti, J., *Experimental Animal Physiology*, Hold Rinehart and Winston, New York, 1969, p. 49.
7. Gupta, D., *Ind. J. Exp. Biol.*, 1967, 5, 243.
8. Dunn, A. and Arditti, J., *Experimental Animal Physiology*, Holt, Rinehart and Winston, New York, 1969, p. 50.
9. Weichselbaum, T. E., *Am. J. Clin. Path.*, 1946, 7, 40.
10. Calkins, E., Taylor, I. M. and Hastings, A. B., *Am. J. Physiol.*, 1954, 177, 211.
11. Flink, E. B., Hastings, A. B. and Lowry, J. D., *Ibid.*, 1950, 163, 598.
12. Case, R. B., Nasser, M. G. and Crompton, R. S., *Am. J. Cardiol.*, 1969, 24, 766.
13. Purshottam, T. and Ghosh, N. C., *Am. J. Physiol.*, 1973; 225, 801.

OCCURRENCE OF NSUTITE (GAMMA-MnO$_2$) IN THE MANGANESE ORES OF SANGUEM DISTRICT, GOA, INDIA

THE area around Sanguem (15° 00′ 00″ and 15° 21′ 16″ N and 74° 04′ 00″ and 74° 15′ 00″ E) District of Goa is one of the important manganese mining areas of India. Fermor[2] (1909) and Dunn[1] (1942) were the earliest workers to study the area. A summary of their work is given by Pascoe[4] (1965). Majumdar[3] (1965) studied the mineralogy of the iron and manganese ores and has reported the presence of pyrolusite, cryptomelane, braunite and manganite from these ores. The present note reports the occurrence of nsutite, which has hitherto not been reported from this area.

Detailed field investigations of the manganese ores of Sanguem area have shown that the deposits are entirely restricted to the phyllites of the Dharwar Supergroup (Archaean). The ore is found as boulders and concretions or as pockets and lenses of varying dimensions in the lateritised phyllites, below which the phyllite is seen altered to lithomarge. The zone of lithomarge is followed downwards by a zone of wad of varying thicknesses and this, at depth, usually grades into a zone of powdery ore. This sequence constitutes the secondary deposits, in which nsutite is found. Rarely below the zone of powdery ore a parent rock rich in manganese is seen.

The manganese ore samples collected from different mines were subjected to ore microscopic examination, gravimetric thermal analysis, differential thermal analysis and X-ray analysis. These studies reveal the presence of braunite and jacobsite which are the primary minerals and pyrolusite, manganite, psilomelan and nsutite as the secondary minerals.

Under the ore microscope, nsutite is white to creamish in colour and consists of fine dusty looking aggregates or felted masses of fine delicate needles. The dusty aggregates appear nearly isotropic. Such aggregates sometimes show shrinkage cracks due to dehydration. The needle-like crystals show distinct anisoropism from grey to dark grey without development of colour and with more or less undulose extinction. Although no confirmatory etch test could be made due to its very fine grained nature, the mineral has been conclusively identified as nsutite by X-ray diffraction studies. It occurs as fine needles replacing psilomelane filling the interspaces of crushed fragments of pyrolusite (Fig. 1) or quartz or rock gangue. It also occurs forming the outermost layers on colloform psilomelane replacing it along the banding or grain boundaries or even irregularly. Sometimes, veins of nsutite are found to traverse rock fragments, which are enclosed in the base of nsutite. Presence of nsutite in secondary deposits forming outermost layers on colloform bands indicates that it has been formed from colloidal sols.

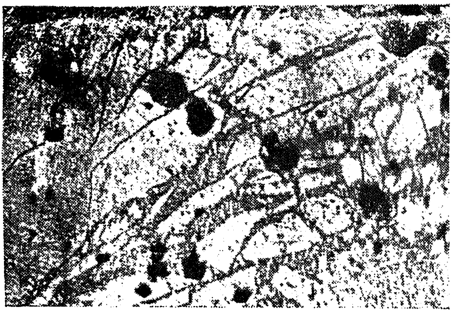

FIG. 1. Pyrolusite is light grey in colour and fractured. The fractures are filled with nsutite (× 30).

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1. Dunn, J. A., "Manganese ore," *Geol. Surv. Ind. Mem.*, 1942, 76, 53.
2. Fermor, L. L., "Manganese ore deposits of India," *Ibid.*, 1909, p. 37.
3. Majumdar, A. K., "Mineralogy of the iron and manganese ores of Sanguem district of Goa," *Mineral Wealth*, 1965, 1, 14.
4. Pascoe, E. H., *A Manual of Geology of India and Burma*, Government of India Press, New Delhi, 1965, p. 485.

CHANGES IN THE ALKALINE PHOSPHATASE ACTIVITY OF OVARIES DURING OVULATION INDUCED BY PITUITARIES IN THE FROG, *RANA CYANOPHLYCTIS* SCHNEIDER

DURING the course of our investigations on the annual variations in certain bio-chemical constituents of ovaries in the frog, *Rana hexadactyla*, a marked increase in the alkaline phosphatase activity was found to correlate with the vitellogenic processes occurring in the oocytes during the breeding season. As the oocytes became gravid the activity decreased significantly. This reduction in the enzyme activity was, also, found to correlate with a similar depletion in the ascorbic acid content of ovaries. It was, therefore, suggested[1] that the changes in the alkaline phosphatase activity of ovaries might be due to the release of gonadotrophins from the pituitary. However, experimental evidence in support of this presumption was inadequate. The ovaries in *R. hexadactyla* would regress soon after the breeding was over towards the end of the south-west monsoon months (June–September). Hence this investigation was undertaken in a related species namely *Rana cyanophlyctis*, which would maintain the gravidity of ovaries round the year[2], as an attempt to study the gonadotrophin action on ovaries at a time when the synthesis and/or release of these hormones were considered to be at their minimum such as the post-breeding season[3].

The breeding season of *R. cyanophlyctis* would commence by June and extend upto the end of September[2]. Adult females weighing 30–38 g were collected from the vicinity of Mysore city (India) during October and maintained separately in aerated aquaria at 25° ± 1° C. They were induced to ovulate with homoplastic pituitaries which were collected from females of similar weight range. The pituitaries were homogenized in distilled water in the proportion of five glands per ml and one ml of this homogenate was administered intraperitoneally into the treated ones[2]. Frogs receiving an equal volume of distilled water served as the controls. The autopsy was carried out at intervals after the treatment, as shown in Table I. Appropriate amounts of the ovarian tissue were weighed and used for the estimation of the enzyme activity. The procedures employed were those of Kind and Macchi[4] for the extraction and Fiske and Subba Rao as cited by Hawk *et al.*[5] for the estimations. Ovaries from a group of non-gravid females were also included for a comparison. The readings were taken from Klett-Summerson photoelectric colorimeter.

It may be observed from Table I that neither the control frogs nor the treated ones autopsied at 2 hours after the pituitary stimulation release eggs from the ovary. The release of eggs commences by about 4 hours and thereafter a progressive increase in the number of animals releasing eggs is noticed. Two distinct phases, namely an ovarian phase from 0 to 12 hours during which ovary releases gravid eggs (ovulation) and an oviductal phase from 12 to 16 hours during which the eggs pass through the oviducts (spawning), may be recognized. The pituitary stimulation brings about a rapid decrease in the alkaline phosphatase activity of ovaries (Table II)

TABLE I

Response of ovary to the pituitary stimulation and ovulation in Rana cyanophlyctis

Groups	Treament	Weight range (g)	Number used	Number of frogs showing eggs in			Degree of response*	Per cent spawned
				Body cavity	Oviducts	Aquaria		
A	Control	30–34	10	Nil	Nil	Nil	Nil	Nil
B	Treated 2 hours after	30–32	9	,,	,,	,,	,,	,,
C	Treated 4 hours after	30–37	9	4	3	1	+	11
D	Treated 8 hours after	30–36	10	9	9	5	+++	50
E	Treated 12 hours after	30–34	8	1	4	5	+++	62
F	Treated 16 hours after	30–38	12	Nil	4	12	+++	100

* + = 1 to 10 eggs released
++ = 10 to 50 eggs released
+++ = 50 or more eggs

TABLE II

Changes in the alkaline phosphatase activity of ovaries in Rana cyanophlyctis

Group	Treatment	Number used	Alkaline phosphatase activity expressed as μg 'P' liberated at 37° C per g wt. of ovary
	Non-gravid untreated	11	2198 ± 267·7
A	Control Distilled water	10	1717·3 ± 171·0
B	Treated: 2 hours after pit.	9	1442·6 ± 150·5
C	4 hours after pit.	9	954·9 ± 119·3
D	8 hours after pit.	10	1006·6 ± 91·8
E	12 hours after pit.	8	1073·0 ± 95·3
F	16 hours after pit.	12	1343·0 ± 14·6

Alkaline phosphatase activity.

A *vs.* B $t = 1{\cdot}201$ $P > 0{\cdot}2$
A *vs.* C $t = 3{\cdot}657$ $P > 0{\cdot}001$
A *vs.* D $t = 3{\cdot}667$ $P > 0{\cdot}001$
A *vs.* E $t = 3{\cdot}331$ $P > 0{\cdot}001$
A *vs.* F $t = 1{\cdot}989$ $P > 0{\cdot}05$

and it is found to be significant by about 4 hours after the treatment ($P > 0\cdot001$). This decrease is maintained upto 12 hours after which the activity registers a recovery. Non-gravid ovaries record higher levels of activity than the gravid controls.

The action of gonadotrophic hormones on the ovary in lower vertebrates is relatively less understood when compared with that of mammals. The fact that the pituitary induced ovulation is blocked by actinomycin, puromycin and cyclohexamide, suggests that the action of gonadotrophins may be associated in the synthesis of *m*RNA and proteins[5-9]. The activity of alkaline phosphatases is generally associated with the growth and differentiation of diverse animal tissues[10,11]. A rise in the activity is reported to occur during certain stages in the development of the oocytes in *Anabas scandens*[12]. The changes observed in the activity of these enzymes during the present investigation seem to represent the rapid cytomorphological differentiation which the oocytes are undergoing due to the action of the pituitary homogenates. Thus, the levels of this enzyme activity may be taken as a measure of the growth and maturation of oocytes in the ovary due to the action of gonadotrophins from the pituitary in Anura.

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---

1. Gopalakrishnan, M., *Doctoral Thesis*, University of Mysore, Mysore (India), 1974.
2. Ramaswami, L. S. and Lakshman, A. B, *Proc. Nat. Inst. Sci. Ind.*, 1959, 25, 68.
3. Oordt, P. G. W. J. van, Dongen, W. J. van, and Lofts, B., *Z. Zellforsch.*, 1968, **88**, 549.
4. Kind, C. A. and Macchi, M. E., *Jour. Cell. Comp. Physiol.*, 1952, 39, 153.
5. Hawk, P. B., Oser, B. L. and Summerson, W. H.. *Practical Physiological Chemistry*, McGraw-Hill, Book Co., Inc., New York, 1954, p. 637.
6. Dettlaff, T. A., *Jour. Embryol. Expt. Morph.*, 1966, 16, 183.
7. Brachet, J., *Expt. Cell. Res.*, 1967; **48**, 233.
8. Shuetz, A. W., *Jour. Exptl. Zool.*, 1967, 166, 347.
9. Brachet, J., Hanocqu, F. and Gansen, P. van, *Developmental Biol.*, 1970, 21, 157.
10. Leathem, J. H., *Ann. N.Y. Acad. Sci.*, 1959, 75, 463.
11. Gangadhara, N. S. and Ramaiah, T. R., *Jour. Zool.*, 1969, 10, 152.
12. Gopal Dutt, N. H. and Govindan, P., *Curr. Sci.*, 1968, 37, 468.

ANABOLIC STEROIDS INCREASE THE PROTEIN CONTENT OF POTATOES

Potato proteins have been adjudged to possess the highest biological value among plant proteins[1,2]. But the concentration of proteins in the tubers is so low that the resultant high starch-to-protein ratio militates against the potato being an efficient source of proteins in human diet; if the protein content were increased, more proteins per joule could be supplied, promoting its role as a balanced food.

Although the variability of protein content among *S. tuberosum* cultivars is quite wide[3], offering scope for increasing the protein content through hybridization techniques, results to date[4] have led to the view that combining high protein content with high bulk productivity holds little promise. Among other means of enriching the tubers with respect to proteins, nitrogenous fertilizers act most potently, whilst reports of the beneficial effects of 2, 4–D, simazine, maleic hydrazide, etc., have yet to receive thorough confirmation; Ora Smith has reviewed the work relating to the various factors that influence protein content in potatoes[4]. An improvement in the efficiency of nitrogenous fertilizers in respect of both the productivity and the protein composition of tubers is of great relevance in view of the current fertilizer price trends.

Androgens are known to promote nitrogen retention by increasing protein synthesis and decreasing the rate of amino acid catabolism in animal systems[5,6]. A number of synthetic anabolic steroids with varying anabolic-to-androgenic activity ratios are presently available commercially[7]. The present study relates to an investigation on the effect of such drugs on the plant system.

Pot culture experiments were conducted during three seasons, beginning in 1974, to find out if synthetic anabolic steroids could help to improve assimilative fixation of foliar-applied urea in potatoes. Each experiment comprised of five treatments, described in Table I, in an eight-fold replication. A 500 ppm stock solution of

TABLE I

Effect of anabolic steroid on the assimilation of foliar-applied urea

Treatments	N in leaves % dry wt.	Fresh tubers: Dry matter %	Fresh tubers: Total nitrogen %	Fresh tubers: Protein-N content %	Fresh tubers: Yield of tubers g per pot
1. Control	3·8	18·3	0·30	0·18	117
2. Foliar spray with 5 ppm methandienone solution	4·0	18·4	0·33	0·20	160
3. Soil application of urea @ 100 kg N/ha	5·1	19·5	0·44	0·28	352
4. Foliar spray with 2% urea solution	5·3	19·3	0·42	0·29	340
5. Foliar spray with a solution 2% in urea and 5 ppm in methandienone	7·2	19·3	0·58	0·39	359
C.D. at 5% level	1·7	3·4	0·14	0·08	65

methandrostenolone (methandienone)[7] the a.i. in the patent formulation, "ANABOLEX" (Cipla Laboratories, Bombay) was prepared in ethanol and dispensed as required. A placebo, *i.e.*, "ANABOLEX" tablets exhaustively extracted free of the a.i. with chloroform and prepared similarly, was used in the control treatment. The first foliar spray @ 600 litres/ha was applied to two month old cv. Kufri Jyoti plants, followed by three further sprays at fortnightly intervals. Homologous leaves[8] were collected a week after the third spray, washed free of spray residues and analysed for total nitrogen. Plants were harvested three weeks after the last spray and the fresh tuber yields were recorded. Samples of fresh tubers from each experimental pot were analyzed for total N and protein-N contents employing rapid procedures[9,10]. Data for the three seasons were combined and a pooled analysis of variance was performed, as it was observed that the responses to the treatments were consistent from season to season and the experimental errors were homogeneous[11]. The results are presented in Table I.

Two conclusions could emerge from a scrutiny of the results (*a*) the effect of the anabolic steroid in increasing the incorporation of foliar-applied urea into nitrogenous constituents of various plant tissues, especially as proteins in the tuber, is quite significant; and (*b*) foliar-applied urea is as efficient as the conventional soil application of larger doses of the fertilizer, both with respect to the bulk yield responses and the nitrogen uptake.

The mechanism of action of anabolic steroids has not been conclusively elucidated yet, although certain guiding principles based on pharmacokinetics are understood to hold the key to the observed effects[12]. We are therefore pursuing our studies in this direction with labelled compounds to unravel the mechanisms that come into play in the enrichment of potato tubers with protein through the drug action and to evaluate the nutritive quality of such enriched tubers.

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1. Kofranyi, E., *Melsunger Med. Mitt.*, 1972, 46 (Suppl. 1), 15; *Chem. Abstr.*, 1973, 78, 83102.
2. Kaldy, M. S., *Econ. Bot.*, 1972, 26, 142.
3. Swaminathan, K. and Pushkarnath, *Indian Potato Jour.*, 1962, 4, 76.
4. International Potato Centre, Lima, Peru, *Report of the Potato Quality Planning Conference*, 1975.
5. Kenyon, A. T., *Biol. Symposia*, 1942, 9, 11.
6. Wilkins, L. and Fleischman, W. J., *Clin. Endocrinol.*, 1946, 6, 382.
7. Mayers, F. H., In: *Rev. Med. Pharmacol.*, Lange Medical Publishers, Los Altos, California, 1970.
8. Chapman, H. D. (Ed.), *Diagnostic Criteria for Plants and Soils*, University of California Press, 1966.
9. Swaminathan, K. and Sud, K. C., *Anal. Biochem.*, 1976, 74, 260.
10. —, — and Hari Kishore, *Indian J. Exp. Biol.*, 1973, 11, 63.
11. Panse, V. G. and Sukhatme, P. V., *Statistical Methods for Agricultural Workers*, Indian Council of Agricultural Research, New Delhi, 1961.
12. Laurence, D. R., *Clinical Pharmacology*, Churchill Livingstone, Edinburgh, 1974.

IS THERE A RACIAL VARIATION IN ALPHA-FETOPROTEIN IN AMNIOTIC FLUID ?

ALPHA-FETOPROTEIN (A.F.P.) is a serum protein specific to the fetus. Increased levels of A.F.P. occur in the maternal serum and the amniotic fluid in pregnancies where the fetus has a neural tube defect like meningomyelocele and anencephaly, and other congenital malformations. This has provided a useful method for the prenatal diagnosis of many serious malformations.

TABLE I

Comparison of A.F.P. values in amniotic fluid in Indian and British women

Gestation (weeks)	No.	Indian A.F.P. mean	Women (ug/ml) S.D.	No.	British A.F.P. mean	Women[4] (ug/ml) S.D.	'*t*' value	'*p*' value
13–14	3	15·1	8·9	38	20·2	8·5	0·97	N.S.
15–16	19	12·2	4·2	61	15·6	5·9	2·74	< ·05
17–18	33	8·5	3·8	51	13·8	6·4	4·77	< ·001
19–20	43	6·4	2·9	18	6·9	3·6	0·52	N.S.
21–22	20	4·3	1·8	12	5·5	2·9	1·22	N.S.
23–24	11	2·1	2·9	12	2·7	2·1	0·58	N.S.

Shapiro and colleagues reported an apparent racial variation in A.F.P. in maternal serum[1]. For nine Asian women at 11 weeks and 15 women at 16 weeks the A.F.P. values were significantly lower than those for Caucasian women with a similar gestation. Watson and Pow, however, observed serum A.F.P. values in 53 Indian or Pakistani mothers to be identical with those of the Caucasian majority[2]. We have assayed A.F.P. in 151 samples of amniotic fluid from 147 pregnant women in Delhi. In 75 women the amniotic fluid was obtained before termination of pregnancy and in 72 amniocentesis was done for diagnostic purposes. The method used for the assay was crossed immunoelectrophoresis[3]. The anti-serum was obtained from a commercial source (Dakopatts), and the standard was the W.H.O. reference preparation 72/225.

Table I compares the values of A.F.P. in amniotic fluid for Indian women with those for Caucasian women in U.K. No significant differences are observed except for gestation of 15–16 and 17–18 weeks. The values are lower for Indian women. However, the reference standards used in Brock's study were different (Abelev and Nishi). The serum estimated by Brock to contain 20 μg/ml of A.F.P. was found to have 17·2 μg/ml of A.F.P. by us. The lower values in our study are probably explained by the different reference standards.

A similar comparison was made wtih the values reported for American women[5]. W.H.O. reference standard (72/225) was used in both studies. The A.F.P. values for the various gestational groups were similar except for 20–21 and 22–23 weeks' gestation (4·7 and 3·5 μg/ml in our study *vs* 6·5 and 5·9 μg/ml in Ainbender and Hirshhorn's study).

Thus, the variation in the A.F.P. values in the amniotic fluid in Indian and Caucasian women is not observed uniformly at all periods of gestation. We conclude that there are no racial variations in the amniotic fluid–A.F.P. values.

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1. Shapiro, L. R., Skinner, L. G.; Phillips; H. V. and Whitfield, C. R., *Lancet*, 1975, ii, 1142.
2. Watson, D. and Pow, M., *Ibid.*, 1976; i, 1015.
3. Brock, D. J. H. and Sutcliffe, R. G., *Ibid.*, 1972, ii, 197.
4. —, Scrimgeour, J. R. and Nelson, M. M., *Clin. Genet.*, 1975, 7, 163.
5. Ainbender, E. and Hirschhorn, K., *Lancet*; 1976, i, 597.

BARK AND SCLEREID STRUCTURE IN *DRIMYS PIPERATA* HOOK.

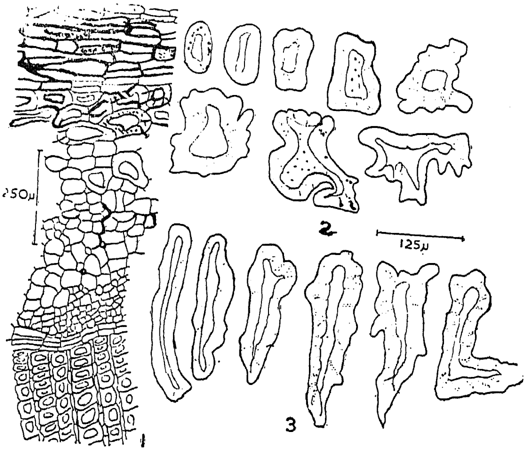
Drimys and other genera of Winteraceae have attracted the attention of several plant anatomists since the wood is vesselless and the reproductive structures show a series of primitive tendencies[1–4]. Occurrence of bundle caps and groups of thick walled stone cells mixed with a few fibres is recorded in the case of winter bark, which is of medicinal importance belonging to *D. winterii*.[5,6] While studying the bark structure in *D. piperata*, regular sclereids, in addition to stone cells are observed. In view of the importance of this taxon, the bark and sclereid structure is briefly reported here.

D. piperata, the only Asiatic species, occurs in Philippines, Borneo and Celebes[7]. The material for the present study was collected in the mossy forests of Mt. Kinabalu at 10,500 ft. and the plants are easily identified by their aromatic leaves with red petioles[8]. Customary methods were followed to obtain sections and macerations[9].

The bark surface is smooth, ash grey in colour and 2–3 varieties of lichens grow on the stems. As seen in transections, the outer bark consists of 10–12 layers of cork cells whose cell walls show uniform thickening and the layers are evenly arranged. Tannin and other cell contents are commonly present. Inner to the cork region, there are 3–4 layers of large, thick walled scleried cells. Both these regions organise the outer bark. Following the sclereid zone is the parenchymatous region that forms the inner bark and inner to this secondary phloem, vascular cambium and secondary xylem are distinctly seen (Fig. 1).

As seen in the macerated preparations the majority of the sclereids (nearly 60%), belong to brachysclereid type and the cell shape is variable (Fig. 2). The bigger sclereids have an undulating outline due to small, round outgrowths at different points. Based on 100 measurements, they are, on an average, 85 μ long, 43 μ wide at the broadest region of the cells and the range being $110 \times 65 \mu$ to $55 \times 33 \mu$. Majority of the sclereids possess thick lamellated wall with simple pits, some of them branched.

The second type of sclereids is basically elongated, with one end being wider than the other (Fig. 3). Small out growths are common. On an average they measure 187 μ long and 57 μ wide, the range being $264 \times 88 \mu$ to $154 \times 44 \mu$. Irrespective of the variations in their shape, the sclereids show thick lamellated wall with simple pits and distinct cell lumen. A few of them show L or V-shaped outline, with one arm being shorter than the other. These are the idiosclereids similar to those reported in other taxa[10–13]. Essentially the sclereid cell displays a linear outline, gradually tapering towards one end with a few outgrowths. The foliar sclereids are reported in species of *Bubbia*, *Drimys* and *Trochodendron*. In the first two genera they are polymorphic and mostly conform to astrosclereid type[5]. In *Trochodendron* both fusiform and ramified types are common[14]. The idiosclerieds described presently in *D. piperata* are distinctly different from the above types.



FIGS. 1–3. *Drimys piperata*. Fig. 1. T. S. bark showing the outer bark with sclereid zone, inner bark, secondary phloem and xylem with vascular cambium in between. Figs. 2, 3. Branchy and idiosclereids showing variations in cell size and form, arranged in increasing order of complexity.

I am thankful to Mr. R. Tay for his technical help.

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1. Bailey I. W. and Thomspon W. P., *Ann. Bot.* 1918, **32**. 503.
2. —, *Amer. Jour. Bot*, 1944, **31**, 421.
3. Cheadle, V. I., *Phytomorphology*, 1953, **3**, 23.
4. Bailey, I. W. and Nast, C. G., *Jour. Arn. Arb.* 1945, **26**, 37.
5. — and —, *Ibid.*, 1944, **25**, 342.
6. Metcalfe, C. R. and Chalk, L., *Anatomy of the Dicotyledons*, Oxford Univ. Press, Oxford, 1950.
7. Smith, A. C., *Jour. Arn. Arb.*, 1943, **24**, 119.
8. Gibbs, L. S., *Jour. Linn. Soc.* (*Bot.*), 1914, **42**, 1.
9. Sass, J. E., *Botanical Microtechniqe*, Iowa University Press, Iowa, 1951.
10. De Roon, A. C., *Acta Bot. Neerl.*, 1967, **15**, 585.
11. Haliburton, B. W., Glasser, W. G. and Byrne, J. M., *Bot. Gaz.*, 1975, **136**, 219.
12. Mia, A. J., *Amer. Jour. Bot.*, 1964, **51**, 78.
13. Rao, T. A., *Jour. Ind. Bot. Soc.*, 1951, **30**, 28.
14. Foster, A. S., *Jour. Arn. Arb.*, 1945, **26**, 155.

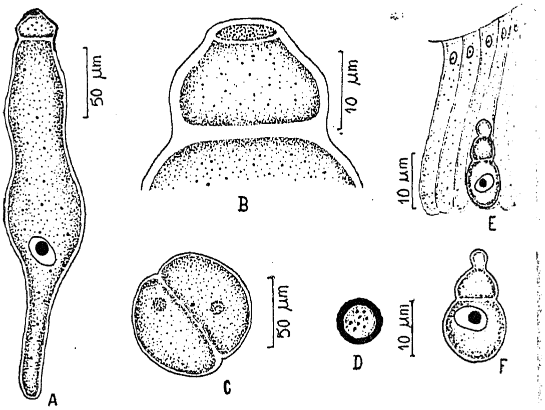
REPORT ON THE LIFE-CYCLE OF THE GREGARINE, *STENOPHORA OZAKII* HUKUI 1952

THE gregarine, *Stenophora ozakii*, was first described[1] in Japan in the millipede, *Orthomorpha gracilis* (Koch). This gregarine was considered new only from the observations based on sporont stages. In the present communicaton, the author has described the different stages in the life-history of the same gregarine infecting about 25% *Chondromorpha kelaarti* Humb and 33% *C. severini* Silv. millipedes collected from the Karnatak University Campus, Dharwar, India.

The Sporont.—The sporont (Fig. 1 A) has an elongated (100-300 µm) body with its maximum width (45–85 µm) either in the middle of the deutomerite or at the shoulder. The deutomerite and the protomerite are separated by an ectoplasmic indentation. The protomerite is usually bell-shaped (Fig. 1 B) and rarely shows slight variations. It measures 14 µm × 19 µm. In majority of sporonts, its length is about 1/12th of the total body length (PL: TL ratio, 1 : 12). The endoplasm is fine and granular. The densely stained area at the apex of the protomerite appears to be the congregation of the reminiscence of the epimerital myonemes. The deutomerite is elongated and is swollen at 2/3rds from the anterior end. It suddenly tapers to a narrow cylindrical form terminating into a blunt end posteriorly. In greater number of sporonts, the width in the swollen region is about 3 times that of the protomerite (PW: DW ratio, 1 : 3). The endoplasm within it is uniformly granular. The nucleus is ellipsoidal (15–25 µm × 10–18 µm) with an eccentrically situated spherical (2-3 µm in diameter) karyosome.

Life-history Stages.—The newly formed gametocyst (Fig. 1 C) is oval in shape. During the course of development it becomes spherical and measures 85–95 µm in diameter. Such cysts mature on the fifth day af their development in moist chamber and release sporocysts by simple rupture. The mature sporocysts (Fig. 1 D) are spherical (7–8 µm in diameter) in shape with relatively thick (2 µm) cyst wall enclosing eight sporozoites. The transverse sections of the proventriculus of the gut of the millipede showed intra-epithelial trophic forms (Fig. 1 E). It has a dome-shaped hyaline epimerite, globular protomerite and an oval deutomerite enclosing an ellipsoidal nucleus. The epimerite is retained in the lumen dwelling cephalonts (Fig. 1 F) till they reach the length of about 75 µm. An oval deutomerite of the trophic forms, in the course of development, becomes elongated and attains the shape as in sporonts. Likewise, the globular protomerite becomes bell-shaped as shown in Fig. 1 A.

This gregarine species seems to have stimulated great deal of interest in the earlier workers[1,2,4]. *Stenophora nematoides* was first reported[4], from the millipede *Strongylosoma italicum* Latz., to be having polymorphic forms, *viz.*, "constriction type" and "nematoid type". Later, the "constriction type" was regarded[2] as an early developmental stage in the process of formation of an adult ("nematoid type") sporont. However, based on the morphological features and stainability of specimens, these polymorphic forms have been considered[1] as two distinct species: the "nematoid type" as *Stenophora nematoides*[4] and the "constriction type" as *Stenophora ozakii*.



FIGS 1 A–F. *Stenophora ozakii* Hukui. A. Mature sporont. B. Enlarged protomerite. C. Freshly-formed gametocyst. D. Sprocyst with thick cyst wall. E. Cephalont about to emerge from the epithelial cell. F. Lumen dwelling cephalin.

The gregarine specimens observed in this study are identical in many respects with the "constriction type" described by the earlier workers[2,4] and also with *Stenophora ozakii*[1] except for slight variations in respect of deutomerite shape: some forms were encountered with maximum width at the anterior 1/3rds, in the middle of the body and also at 2/3rds from the anterior end. Such variation in the deutomerite form is likely to appear in the process of movement of the gregarine. Hence, the gregarine species is regarded as *Stenophora ozakii*[1].

These supplementary observations, apart from revealing the life-cycle of the gregarine, confirm the validity of the species under the genus *Stenophora*[3].

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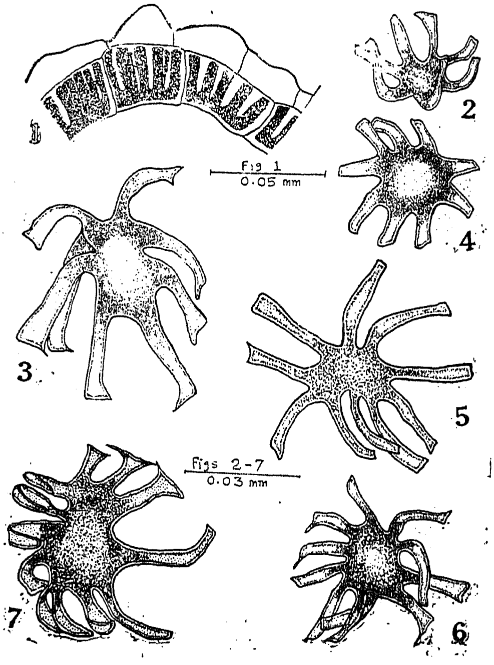
---

1. Hukui, T., *Zool. Mag., Tokyo*, 1952, **62**, 91.
2. Karandikar, K. R. and Rodgi, S. S., *J. Univ. Bombay*, 1955, **24** (3), 11.
3. Labbé, A., "Sporozoa," In *Das Tierreich*, 1899, **5**, 1.
4. Léger, L. and Duboscq, O., *Arch. Protistenk.*, 1904, **4**, 335.

THE ROLE OF ENDOTHECIUM IN THE IDENTIFICATION OF UMBELLIFERS

In the anther the endothecial cells develop characteristic fibrous thickenings[1,2]. Sehgal[3] observed that these thickenings are attached on the inner tangential walls and they appear like a 5–7 ribbed inverted umbrella in the lateral and surface view. This interesting observation prompted us to examine the smear preparations of the endothecium in the various genera of the family at our disposal.

There is a stellate thickening in each endothecial cell in all the seventeen genera described here. They are accommodated in the cells in such a way as to simply appear fibrous as in most of the angiosperms (Fig. 1). However, the central portion of the star is spread over in the form of a plate on the inner tangential wall and 7–12 rays (Figs. 2–7) diverge, extending up to the outer tangential wall of the cell. The size of the star, the diameter of the plate and the number of rays to a star appear to be characteristic for a genus.



Figs. 1–7. Endothecial thickenings in Umbelliferae. Fig. 1. T.s. *Psammogeton biternatum,* a portion of anther wall, endothecium. Fig. 2. *Bupleurum* sp., whole mount of star-shaped thickening from an endothecial cell, 7 rays. Fig. 3. *Sanicula europea,* same, 8 rays. Fig. 4. *Chaerophyllum aromaticum,* same, 9 rays. Fig. 5. *Foeniculum vulgare,* same, 10 rays. Fig. 6. *Anthriscus sylvestris,* same, 11 rays. Fig. 7. *Peucedanum graveolens,* same, 12 rays.

The rays are larger in *Sanicula europea* L., *Astrantia major* L., *Seseli indicum* W.A., *Foeniculum vulgare* Mill., *Peucedanum graveolens* L., *Laserpitium peucedanoides* L., and *Daucus carota* L.; medium sized in *Chaerophyllum aromaticum* L., *Anthriscus sylvestris* (L.) Hoffm., *Smyrnium perfoliatum* L., *Angelica* sp., *Angelica archangelica* L., *Trachyspermum ammi* L., *Ferula communis* L., and *Cuminum cyminum* L.; smaller in *Eryngium amethystinum* L., *Psammogeton biternatum* Edgw., *Bupleurum* sp., and smallest in *Coriandrum sativum* L. The diameter of the plate of the star is larger than the length of the ray in *Eryngium amethystinum* L., *Chaerophyllum aromaticum* L., *Bupleurum* sp., *Foeniculum vulgare* Mill., *Peucedanum graveolens* L., *Angelica archangelica* L., and *Daucus carota* L.; smaller in *Sanicula europea* L., *Anthriscus sylvestris* (L.) Hoffm., *Coriandrum sativum* L., *Seseli indicum* W.A. and *Angelica archangelica* L.; and both of them are almost of equal size in *Astrantia major* L., *Psammogeton biternatum* Edgew., *Smyrnium perfoliatum* L., *Trachyspermum ammi* L., *Ferula communis* L., *Laserpetium peucedanoides* L. and *Cuminum cyminum* L.

It is concluded that the size of the star and its rays may provide diagnostic characters for the identification of at least the genera of the Umbelliferae.

We are grateful to Professor J. Heslop-Harrison, F.R.S., Ex-Director, Royal Botanic Gardens, Kew, England, for the material of many umbellifers, and Dr. D. Singh, Head of the Department of Botany, University of Rajasthan, for laboratory facilities.

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1. Dhakre, J. S., *Jour. Indian bot. Soc.*. 1968, 47, 13.
2. —, *Agra Univ. Jour. Res. (Sci.)*, 1969, **18**, 5.
3. Sehgal, C. B., *Proc. nat. Sci. India*, 1965, **31**, 175.

AMYLASE SECRETION BY SEED-BORNE FUNGI OF *SORGHUM* VARIETY CSH-1

Deterioration of grains is usually attributed to the action of extracellular enzymes secreted by seed-borne fungi or bacteria[1]. A study of seed-borne fungi of Jowar (*S. vulgare*, variety CSH–1) revealed constant association of certain fungi with deteriorating grains. Experiments were conducted to assess the ability of these fungi for the secretion of amylase.

Standard blotter test was employed for the determination of seed-mycoflora. For enzyme production a basal salt medium (0·25% KNO_3, 0·1% KH_2PO_4, 0·05% $MgSO_4 . 7H_2O$) containing either 1% soluble starch or Jowar meal (JM) from variety CSH-1 was used at pH 5·4. Twenty-five ml of medium was seeded with 0·5 ml of spore suspension prepared by harvesting the spores from five day old PDA slant cultures with 10 ml of sterile water. The cultures were incubated for five days on a rotary shaker (150 rpm) at 27 ± 1° C. Culture filtrate obtained by filtration through Whatmann No. 40 filter paper was used as the crude enzyme preparation. Amylase activity was assayed by determining the increased amount of reducing sugars using 3, 5-dinitrosalicylic acid (DNSA) reagent[2]. One ml of crude enzyme (culture filtrate) was incubated with 1 ml of substrate (1% soluble starch–BDH in 0·016 M acetate buffer at pH 5·0) in a test tube for 10 minutes at 28 ± 1° C. The reaction was terminated by adding two ml of the DNSA reagent. The tubes were kept in boiling water for 5 minutes, immediately cooled in running tap water and the contents were finally diluted to 20 ml. Blanks were prepared by incubating the starch solution with heat inactivated enzyme in the same manner. Colour intensity was read at 540 nm in Erma AE-II Colorimeter. Maltose released mg/ml of the culture filtrate was calculated form the standard curve prepared using different concentrations of maltose.

Different types of fungi (*Aspergillus niger*, *A. sulphureus*, *Alternaria tenuis*, *Curvularia lunata*, *Fusarium moniliformae*, *Helminthosporium rostratum*, *Mortierella humicola*, and *Rhizopus oryzae*) could be isolated from the surface of the germinating seeds and their incidence ranged from 12–35%. *C. lunata* and *F. moniliformae* were associated respectively with 35 and 22% seeds examined. *A. niger* and *H. rostratum* gave 12% incidence. All these fungi are known to be associated with starchy seeds[3-4]. Jower meal medium was preferred to starch medium by these fungi for amylase production. Maximum amylase activity was shown by *A. niger* (0·75 mg/ml on JM and 0·50 mg/ml on starch) and was followed by *R. oryzae*, *F. moniliformae*, *H. rostratum*, *C. lunata*, *A. tenuis*, *A. sulphureus* and *M. humicola*. *A. tenuis* gave slightly increased amylase activity on starch medium. Increased mycelial growth of moulds was noticed on the medium containing Jower meal. *A. niger* and few other species of *Aspergillus* and *Fusarium* are also known to produce amylase on various media[5-6]. Jowar grains contain proteins and lipids in addition to starch. Superiority of Jowar meal medium to starch medium can be attributed to additional source of nutrients which probably stimulated amylase secretion.

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---

1. Christensen, C. M. and Kaufmann, *Ann. Rev. Phytopath.*, 1965, 7, 69.
2. Peter Bernfeld, In: *Methods in Enzymology*, Eds. S. P. Colowick and N. O. Kaplan, Academic Press, New York, 1955, **1**, 149.
3. Kanaujia, R. S. and Singh, C. S., *Indian Phytopath.*, 1975, **28** (2), 299.
4. Deshpande, K. S. and Wadje, S. S., *Marathwada Univ. J. Sci.* (*B*), 1975 (In press).
5. Chahal, D. S. and Sidhu, R. S., *Labdev. J. Sci. Technol. Part B, Life Sci.*, 1972, **10** (1), 27.
6. Smirnov, V. I., Kostik, F. D., Todirash, V. E., Mazur, L. N. and Mogilenco, L. N., *IZV. Akad. Nauk. Mold. SSR. Biol. Khim. Nauk.*, 1972, **5**, 52.

THE EFFECT OF WEED CONTROL TREATMENTS ON BOTH WEEDS AND *CYMBOPOGON CITRATUS*

Many workers have pointed out the deleterious effect of weeds on yield as they are naturally strong competitors (Singh and Singh[5], 1939; Klingman[4], 1961; King[3], 1966). However, very little work has been done to determine the effects of herbicides on aromatic plants. Marked increase in growth and oil content of rose, *Salvia sclarea*, peppermint and lavender due to herbicidal application was noticed by Kamennobrodskaja[2] (1967). Moreover, during harvesting of *Cymbopogon citratus* some weeds collected with the main crop and affected its quality. Therefore, chemical control methods are highly desirable.

Material and Methods

The present work was conducted on the Experimental Station of Faculty of Agriculture, Tanta University, Kafr-El-Sheikh, Egypt. Seven pre-emergence herbicides, Cobex 1 L, treflan 1 kg, planavin 1 kg, bladex 1 kg, Cotoran 1 kg, perforan 2 L, stomp 2 L per feddan were used in this study. The experiment included eleven treatments. The mentioned seven herbicides were employed in addition to a mixture of half of the recommended doses of cotoran with bladex or planavin, hoeing and the unweeded control. A randomized complete block design with four replications was used.

The clumps of *Cymbopogon citratus* were divided into uniform slips and planted on March 18, 1975, at 50 cm spacing on rows 75 cm apart. The area of each plot was 2 × 3 m. There were 16 plants per each plot. On April 24 the plants were fertilized with 200 kg of each of ammonium sulphate and superphosphate and 100 kg potassium sulphate per feddan (hectare about 2·5 feddan). Irrigation was done regularly. The above-ground parts were cut (10 cm above-ground) on August 2, 1975, and volatile oil

TABLE I

The effect of weed control treatments on both weeds and Cymbopogon citratus

Treatment	Weight of above-ground parts per plant (gm)	Volatile oil in above-ground parts (%)	Volatile oil content per plant (μg)	Dry weight of weeds (gm/plot)
1. Cobex	323·5	0·335	1083	231·0
2. Treflan	250·3	0·341	852	689·1
3. Planavin	241·0	0·338	814	692·5
4. Bladex	305·2	0·350	1068	354·1
5. Bladex + Cotoran	307·5	0·342	1050	306·2
6. Planavin + Cotoran	265·4	0·348	923	656·4
7. Cotoran	318·6	0·336	1071	240·5
8. Preforan	211·9	0·335	709	892·4
9. Stomp	203·7	0·342	697	869·6
10. Hoeing	297·0	0·340	1009	184·9
11. Control	198·5	0·332	658	1029·2
L.S.D. at 0·05	28·3	N.S.	87·1	197·5

content of the fresh above-ground parts was determined by the *Egyptian Pharmacopceia*[1] (1963). All the weeds were collected at the end of the season, dried to constant weight for 24 hours at 90° C, and the dried weights as gm/plot were recorded.

Results and Discussion

Table I shows that the weight of fresh above-ground parts was significantly higher due to the herbicides Cobex, Bladex, Cotoran, a mixture of bladex and Cotoran and hoeing than the other treatments included in this experiment. The treatments included the herbicides perform and stomp and unweeded control gave the lowest weights of fresh above-ground parts. These results obtained might be due to the weed control with Cobex, bladex, Cotoran, mixture of bladex and Cotoran and hoeing. This is confirmed by the lowest dry weight of weeds per plot found in those treatments. This reduced the competitive effects leading to the gain the growth of *Cymbopogon citratus* plants (Singh and Singh[5], 1939; Klingman[4], 1961; King[3]1966). Also this agrees with the findings of Kamennobrodskaja[2] (1967) with different herbicides on rose.

The percentage of the volatile oil in the above-ground parts ranged from 0·332% to 0·350% without any significant differences between the treatments included in this experiment. The volatile oil content per plant followed the same trend as the weight of the above-ground parts. The highest volatile oil content per plant were from the treatments: Cobex, bladex, bladex *plus* Cotoran, Cotoran and hoeing. This result was confirmed by the previous studies by Kamennobrodskaja[2] (1967) using different herbicides on rose, *Salvia sclarea*, peppermint and lavender.

It could be noted from Table I that the dry weights of weeds per plot were generally lower in weeding treatments than those of the unweeded control. Each herbicidal treatment, Cobex, bladex, Cotoran, Ccotoran *plus* bladex and hoeing caused a significant reduction in dry weight of weeds compared with the unweeded control. This result reflected on the yield of the main crop, *Cymbopogon citratus*, as the higher yield the lower the dry weight of weeds.

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---

1. *Egyptian Pharmacopoeia*, 1963.
2. Kamennobrodskaja, V. G., *Hort Abst.*, 1967, 38, 4089.
3. King, L. J., *Weeds of the World. Biology and Control*, Intersci. Publ. Inc.. New York. 1966, pp. 162 and 243.
4. Klingman, G. C., *Weed Control as Science*, John Wiley and Sons, Inc., London, 1961, p. 1.
5. Singh, B. N. and Singh, L. B., *Soil Sci.*, 1939, 47, 227.

CYTO-TAXONOMIC STUDIES IN *CHLOROPHYTUM* KER-GAWL. (LILIACEAE)

SIXTEEN wild species of *Chlorophytum* Ker-Gawl. are reported from India, of which except for four species restricted to North–Eastern India, the rest occur along Western and peninsular India. The centre of dispersal is possibly along Western Ghats where at least 8 species are endemic to the region. Cytological studies, mostly confined to the chromosome studies and karyomorphology have been carried out on eleven wild species by various workers but reports pertaining to *C. attenuatum*[1] and *C. glaucum*[2] are evidently based on misidentification. During the course of a taxonomic revision of the genus, meiotic studies following the normal aceto-carmine squash technique were carried out on 7 species, of which three marked* in the table are first reports. Voucher specimens are deposited at *BSI*.

Chlorophytum borivilianum Sant. and Fernandez, endemic to Maharashtra and readily distinguished from all other species by the tubers arising directly from the base, reveals $n = 8$ which is a first report. Three other species investigated, *viz.*, *C. bharuchae* Ans. *et al.*, *C. laxum* R. Br. and *C. tuberosum* Baker also exhibit $n = 8$ which agree with earlier findings (*cf.* Table I). Meiosis was regular in all the above species except in *C. laxum* where pollen mother cells also exhibited various multivalent associations as $6_{11} + 1_{IV}$, $5_{11} + 1_{V}$ and $4_{11} + 2_{IV}$ besides the normal 8 bivalents. As reported by Naik[1] lagging of chromosomes and the tendency for elimination of one unoriented bivalent was observed in *C. laxum*. All the above four species are diploids with $x = 8$ and well adapted to survive from arid tracts to deciduous forests.

As Panigrahi[3] has clarified, *C. orchidastrum* Lindl. is exclusively African, never occurring wild in India, the binomial wrongly applied in the various floras to the Indian species which is actually referable to *C. nimmonii* Dalz. The present report of $n = 21$ for this species agrees with the findings of Sheriff[4] for the peninsular Indian plants. He had reported under "*C. orchidastrum*". Both *C. glaucum* Dalz. and *C. glaucoides* Blatt., restricted along the Sahyadri ranges, reveal $n = 21$ which are new reports. Incidentally the report[2] of $2n = 16$ for *C. glaucum* collected from Daulatabad (Marathwada) is a misidentification for *C. bharuchae*, so common at Daulatabad but where *C. glaucum* does not occur. All the three species are hexaploids with $x = 7$. In these species a large percentage of meiocytes form various multivalent associations such as $19_{11} + 1_{IV}$, $17_{11} + 2_{IV}$, $15_{11} + 3_{IV}$ and $18_{11} + 1_{IV}$ indicating partial homology between the chromosomes. The evidence is rather meagre to comment on the nature of polyploidy but possibly autopolyploidy is involved.

TABLE I

Sl. No.	Species	Voucher specimens deposited in *BSI*	Earlier reports	Present report
1	*Chlorophytum bharuchae* Ansari *et al.*	Junnar, *Ansari* 88712	$n = 8$; $2n = 16$[5]	$n = 8$
2.	*C. laxum* R. Br.	Junnar, *Ansari* 88778	**$n = 7$; $2n = 14$ (*cf.* Naik[1] for (non-Indian) details)	
			$n = 8$; $2n = 16$ do. (Indian)	$n = 8$
3.	*C. tuberosum* Baker	Shahapur (Thana Dist.) *Billore* 111826	***$n = 8$; $2n = 16$ (*cf.* Sheriff and Chennaveeriah[2] for details)	$n = 8$
4.	*C. borivilianum* Sant. et Fernandez	Matheran, *Ansari* 104944		*$n = 8$
5.	*C. nimmonii* Dalz.	Onda (Goa) *Raghavan* 103377	$n = 21$[4]	$2n = 42$
6.	*C. glaucum* Dalz.	Mahabaleshwar, *Ansari* 67594	..	*$n = 21$
7.	*C. glaucoides* Blatt.	Purandhar, *Rolla S. Rao* 88629; Thana, *Billore* 112909		*$n = 21$

* New reports, ** *cf.* Naik[1] for reports by other workers.

*** *cf.* Sheriff and Chennaveeriah[2] for reports by other workers.

Detailed morphological studies supported by field observations and experimental cultivation suggest that the status of *C. acaule* as a distinct species is doubtful and it may be only a variant of *C. laxum* as hinted by J. D. Hooker in the *Flora of British India*. In *C. acaule*, very few flowers are produced on a sessile corymbose inflorescence hidden beneath the sheathing base of the leaves whereas in *C. laxum* the scape is filiform and flexuous with lax flowers. However, *C. laxum* also exhibits the typical inflorescence of *C. acaule* when exposed to xerophytic conditions and under cultivation both types of inflorescences occur in one and the same plant. This is further supported by both species having $x = 8$. Incidentally, the reporting of $2n = 28$ for *C. attenuatum* by Naik[1] is a misidentification for *C. comosum*, a cultivar (personal communication).

Summing up, barring *C. malabaricum* Baker, *C. breviscapum* Dalz., *C. attenuatum* Baker and *C. undulatum* Wall, the remaining 12 species fall under two series. The series with $x = 8$ is exclusively composed of diploids (5 species) whereas different ranges of polyploidy are seen in $x = 7$ series. The complete absence of polyploids and the greater number of diploids under $x = 8$ series (as against the wide range of polyploids and only one diploid under the $x = 7$ series), the tendency to eliminate one unoriented lagging bivalent in *C. laxum*, coupled with the primitive karyotype in the $x = 8$ series[1] indicate that the basic number of $x = 7$ is advanced and derived from $x = 8$.

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Botanical Survey of India, Western Circle, Poona 1,
February 4, 1977.

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---

1. Naik, V. N., "Chromosomal behaviour and evolutionary trends in *Chlorophytum* (Liliaceae)," *J. Linn. Soc.* (*Bot.*), 1976, 72, 45.
2. Sheriff, A. and Chennaveeriah, M. S., "Karyomorphology of few diploid species of *Chlorophytum*," *Nucleus*, 1972, 15, 39.
3. Panigrahi, G., "*Chlorophytum orchidastrum sensu lat.* (Liliaceae) from Africa and Asia," *Kew Bull.*, 1975, 30, 563.
4. Sheriff, A., "Cytological and cytotaxonomic studies in certain members of Liliaceae," *Ph.D. Dissertation*, Bangalore University, 1967.
5. Naik, V. N., "Cytological studies in *Chlorophytum bharuchae* Ans. Ragh. *et al.*", *Curr. Sci.*, 1974, 43, 161.

POLLEN GRAINS IN THE STYLAR CANAL OF *MORINGA CONCANENSIS* NIMMO

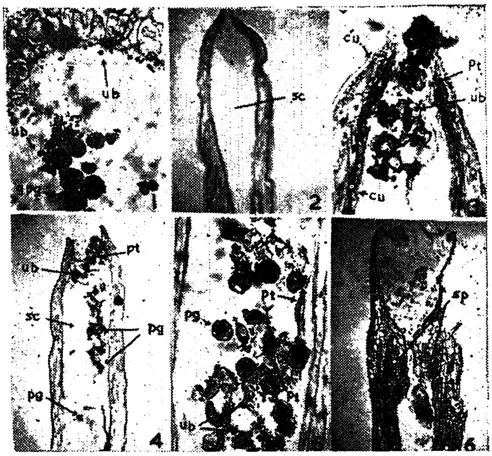
The flowers of *Moringa concanensis*, a chloroembryophyte, are protandrous and have five fertile stamens of unequal height and the anthers dehisce longitudinally forming a 'head'. Abundant pollenkitt is found around the pollen grains which adhere in lumps (Figs. 3, 4). Its presence may also be responsible for the adherence of Ubisch bodies to the pollen grains even after pollination (Figs. 1, 3–5), a feature hitherto unrecorded.

The style and stigma remain below the anthers upto the time of anthesis. The style, however, elongates at a later stage and the stigma comes to lie at a higher level. To start with, the stylar canal, which opens to the exterior, is narrow at the apex (Fig. 2), but attains its maximum development as the gynoecium matures and a clear circular aperture could be observed in the region of the stigma, which is described as truncate (Gamble[2], 1925; Hutchinson[3], 1960; Lawrence[4], 1960), but a critical examination reveals it to be subdentate. After anthesis the pollen falls on the stigma of some of the flowers and as the style slowly elongates pushing its way between the anthers, the pollen mass is gently pressed on the stigmatic surface and consequently it is forced into the stylar canal. Some of the pollen grains slowly descend down on their own weight. Despite careful observations made on 20 plants, no insect agent bringing about pollination could be found. Further, no trace of any 'pollination drop' secreted by the stigma could be detected even after examining hundreds of flowers.

The stigmatic surface is slightly glandular and the outer epidermis is cutinised (Fig. 3). The cells of the transmitting tissue of the stylar canal are elongate and slightly cutinised (Fig. 3). In one section as many as 28 pollen grains could be seen in the stylar canal and some of them had germinated (Figs. 4, 5). Since some preparations showed germinating pollen grains in the anther itself (Fig. 1), too much importance cannot be attached to the place of germination of the pollen grains.

Although there have been some reports on the occurrence of intracarpellary pollen grains in angiosperms, the precise mechanism responsible for this phenomenon has not been satisfactorily explained (Eames[1], 1961, p. 203; Maheshwari[5], 1960, p. 11). It becomes obvious that in *Moringa concanensis*, it is brought about by mechanical forces.

In some preparations, the cells of the stylar canal elongated radially, became meristematic and plugged the passage of the stylar canal (Fig. 6). Such a feature is unknown in angiosperms, although an analogous situation is known in *Gnetum,* where the plugging tissue of the micropylar canal is integumentary in origin but without the meristematic activity of the cells.



FIGS. 1–6. *Moringa concanensis* Nimmo. Fig. 1. T.S. part of dehiscing anther lobe showing abundant Ubisch bodies clinging to the pollen grains, × 1,150. Fig. 2. L.S. part of style showing the stylar canal, × 320. Fig. 3. L.S. apical part of style showing pollen grains and Ubisch bodies, × 1,150. Fig, 4. L.S. part of the style showing pollen grains at various distances, Ubisch bodies and germinated pollen grain, × 320. Fig. 5. L.S. part of the stylar canal showing 23 out of 28 pollen grains (see text). Note the germinated pollen grains and Ubisch bodies, × 1,150. Fig. 6. L.S. style showing the sub-apical plug, × 320.
(*cu,* cuticle ; *gpg,* germinating pollen grain ; *pg,* pollen grain ; *pt,* pollen tube ; *sc,* stylar canal ; *ub,* ubisch bodies ; *sp,* stylar plug.)

A detailed account of the life-history of *Moringa concanensis* will be published elsewhere.

The senior author expresses his sincere thanks to the U.G.C. for an honorarium and to the authorities of A.N.R. College for facilities. Our thanks are also due to Messrs. M. A. Jabbar and D. Lakshmana Rao, for their help in collecting the material and to Dr. (Mrs.) N. Lakshmi, for taking the photographs.

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---

1. Eames, A. J., *Morphology of the Angiosperms,* McGraw-Hill Book Company, New York, 1961.
2. Gamble, J. S., *The Flora of the Presidency of Madras,* London, 1925.
3. Hutchinson, J., *The families of Flowering Plants,* Vol. I, *Dicotyledons,* Oxford, 1960.
4. Lawrence, G. H. M., *Taxonomy of Vascular Plants,* New York, 1960.
5. Maheshwari, P., *7th Seward Memorial Lecture,* Birbal Sahni Institute of Paleobotany, Lucknow, 1960.

QUANTITATIVE ESTIMATION OF THE TOTAL CARBOHYDRATES, PROTEINS AND NUCLEIC ACID CONTENTS IN THE THORACIC GANGLION OF *POTAMON MAGNUM MAGNUM* (PRETZMAN)

THE present investigation deals with the quantitative estimation of the total carbohydrate, protein and nucleic acid contents in the thoracic ganglion of *Potamon magnum magnum.* Very little work has been done in this field and most of our information was obtained from the histochemical studies. It has been shown by Otsu[1] and Otsu and Sonobe[2] (electrophoresis and paper chromatography) that certain chromactivating substances extracted from the thoracic ganglion of the crab, *Eriocheir japonicus* contain cystine and further suggested that these substances are polypeptides containing 10 to 13 amino acid residues, including cystine rich S–S bonds. The existence of chromatophorotropins in the thoracic ganglion of crustaceans has been demonstrated by Smith[3], Enami[4] and Brown[5,6].

The thoracic ganglion was obtained in all the four seasons. Total carbohydrates, proteins and nucleic acids were quantitatively determined by standard methods[7–10].

The results are summarized in Table I.

TABLE I

Material detected	mg/gm fresh weight			
	Summer	Autumn	Winter	Spring
Carbohydrates	0·595	0·577	0·534	0·589
Proteins	0·911	0·915	0·934	0·928
Nucleic acid	0·412	0·466	0·567	0·427

It shows that proteins constitute the major part of the thoracic ganglion. In winter, the ganglionic mass contains more of protein and nucleic acid than in other seasons while the amount of carbohydrates is maximum in summer.

It is difficult to interpret the presence of more material in winter than in other seasons, but it is logical to assume that during active period the demand for secretory material is more and, therefore, there is a depletion of secretory material in the cells.

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1. Otsu, T., *Naturwissenschaften*, 1965, **52**, 87.
2. — and Sonobe, M., *Annot. Zool. Jap.*, 1965, **38**, 127.
3. Smith, R. I., *Biol. Bull.*, 1948, **95**, 169.
4. Enami, M., *Ibid.*, 1951, **101**, 241.
5. Brown, F. A., Jr., *Collecting Net*, 1949, **19**, 8.
6. —, *Biol Bull.*, 1948, **95**, 169.
7. Rutherford, P. P. and Weston, E. W., *Photochemistry*, 1968, **7**, 175.
8. Lowry, O. H., Rosebrogh, N. J., Faw, A. L. and Randall, *J. Biol. Chem.*, 1951, **193**, 265.
9. Cherry, J. H., *Plant Physiol.*, 1962, **37**, 670.
10. —, *Ibid.*, 1963, 38, 440.

CHEMOTAXONOMY OF A FEW MEMBERS OF SCROPHULARIACEAE

SCROPHULARIACEAE include 220 genera and 3000 species (Willis[11], 1966) and some of these are parasitic like species of *Striga* and *Sopubia*. While the members of the family including parasitic species, have received attention from such aspects as of floral anatomy, embryology, palynology and cytology, they have not received much attention from the discipline of chemotaxonomy. The literature on this aspect has been cited by Gibbs[5] (1974). The present paper deals with the chemotaxonomy of *Sopubia delphinifolia* (L.) G. Don., *Striga angustifolia* (D. Don) Sald, *S. sulphurea* Dalz., *S. asiatica* (L.) Kuntze, *S. densiflora* (Bentham) Bentham and *S. gesnerioides* (Willd) Vatble.

The materials of these species for the present study were collected from plants growing wild in the Kakatiya University campus. Using 80% ethanol extracts of entire plants at the time of flowering and fruiting, tests for the following substances were carried out: carbohydrates (Molisch test), saponins, tannins, flavonoids, alkaloids, phenols, indoles (Ehrlich test), leucoanthocyanins, triterpenoids (Noller's test) and, triterpenoids/steroids (Liebermann–Burchard test). Syringin test, Maule test, HCl/Methanol test, cigarette test, hot water test, leucoanthocyanins test 'A' and Aurone test 'A' (Gibbs[5], 1974) were done using fresh stems, leaves and flowers.

The results of the tests are presented in Table I.

It will be seen from the table that all the species gave +ve reactions for carbohydrates, flavonoids, phenols, maule test, cigarette test and hot water test. The last two tests, namely, cigarette and hot water tests could not be carried out for *Striga gesnerioides* since they are leafless. Uniformly negative results were obtained for tannins, triterpenoids, alkaloids, indoles, leucoanthocyanins, syringaldehydes, HCl/methanol test and Aurone test 'A' in the case of all the taxa. The last test was carried out only for the flowers of *Striga sulphurea* which are yellow. Thus all the species exhibit a uniformity in their chemical characters. *Striga gesnerioides* and *Striga densiflora* gave positive reaction for saponins, while the rest of the species gave negative reactions. In case of *Sopubia delphinifolia*, however, the saponin test is doubtfully positive.

TABLE I

Name of the Plant	Molisch test	Saponins	Tannins	Flavonoids	Noller's Test (Triterpenoids)	Alkaloids	Phenols	Ehrlich test (Indoles)	Leucoanthocyanins	Liebermann-Burchard test Triterpenoids/steroids	Syringin test	Maule test	Cigarette test	Hot water test	Leucoanthocyanins test 'A' (Gibbs)	Aurone test 'A'	HCl/Methanol test (Gibbs)
Striga angustifolia	+	−	−	+	−	−	+	−	−	+	−	+	+	+	−	−	−
Striga asiatica	+	−	−	+	−	−	+	−	−	−	−	+	+	+	−	−	−
Striga sulphurea	+	−	−	+	−	−	+	−	−	−	−	+	+	+	−	−	−
Striga gesnerioides	+	+	−	+	−	−	+	−	−	+	−	+			−	−	−
Striga densiflora	+	+	−	+	−	−	+	−	−	+	−	+	+	+	−	−	−
Sopubia delphinifolia	+	?	−	+	?	−	+	−	−	?	−	+	+	+	−	−	−

For Noller's test for triterpnoids all the species gave negative results while *Sopubia delphinifolia* gave a doubtfully positive reaction. In *Striga angustifolia, Striga gesnerioides* and *Striga densiflora*, the Liebermann-Burchard test (triterpenoids/steroids) is positive indicating the presence of steroids in these taxa. Interestingly enough, *Sopubia delphinifolia* gave doubtfully positive results for both these tests. This may seem to indicate the doubtful presence of both triterpenoids and steroids.

From the above discussion, it is clear that all the species under the present study agree closely in their chemical characters. They also resemble broadly the other taxa of Scrophulariaceae in these characters. Further, the available data from disciplines like morphology (Varghese[10], 1967), anatomy (Metcalfe and Chalk, 1950), palynology (Erdtman[4], 1971) and embryology (Davis[3], 1966; Tiagi, 1956[6], 1965[7], 1966[8]; Arekal[1], 1966; Arekal and Raju[2], 1967; Tiagi[9], 1968) also show that the taxa included in the present study resemble the other taxa of Scrophulariaceae.

Our thanks are due to Prof. U. B. S. Swami, Professor of Botany, Kakatiya University, Warangal, for facilities and encouragement. Our grateful thanks are due to Dr. K. Subramanyam for his valuable criticism.

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1. Arekal, Govindappa D., *Proc. Ind. Acad. Sci.*, 1966, 64 **B**, 241.
2. — and Raju, D., *Curr. Sci.*, 1967, 36, 22.
3. Davis, G. L., *Systematic Embryology of the Angiosperms*, John Wiley and Sons, Inc., New York, 1966.
4. Erdtman, G., *Pollen Morphology and Plant Taxonony*. New York, 1971.
5. Gibbs, R. D., *Chemotaxonomy of Flowering Plants*, Vols. I–III, McGill-Queen's University Press, Montreal and London, 1974.
6. Tiagi, B., *Bull. Torrey Bot. Cl.*, 1956, **83**, 154.
7. —, *Can. J. Bot.*, 1965, **43**, 1511.
8. —, *Am. J. Bot.*, 1966, **53**, 645.
9. Tiagi, Y. D., *Acta Bot. Acad. Sci. Hung.*, 1968, **14**, 197.
10. Varghese, T. M., *Agra Univ. J.*, 1967, **15**, 153.
11. Willis, J. C., *A Dictionary of Flowering Plants and Ferns*, Oxford, 1966.

SHORT SCIENTIFIC NOTES

Dissociation Products and Dissociation Energies of $X^1\Sigma$ and $B^1\Sigma$ States of Diatomic MgS

As the diatomic MgS is chemically stable, it is of interest to determine the dissociation energies and dissociation products of the ground and excited states of MgS molecule in understanding the nature of the chemical bond. From the experimental data available on the $X^1\Sigma$ and $B^1\Sigma$ states of MgS[1], potential energy curves have been drawn for the ground and the excited $B^1\Sigma$ states using the RKRV method. These curves have been used to determine the maximum and minimum points of vibration for these two states.

The dissociation energies of these two states have been estimated by fitting the empirical functions[2] of Lippincott, H–H and the electronegativity functions to the true potential curve. The estimated values of the ground $X^1\Sigma$ and excited $B^1\Sigma$ states are 2·4 ev and 2·2 ev respectively. By using the estimated dissociation energies, excited $B^1\Sigma$ state is found to dissociate into an excited Mg (^3P) and an unexcited S (^3P) whereas the ground state ($X^1\Sigma$) dissociation products are written down in the traditional way[2] as an unexcited combination of Mg (^1S) and S (^3P) atoms which are in consistency with the Mullikanes correlation of atomic and molecular states.

One of the authors (M.L.P.) wishes to thank C.S.I.R., New Delhi, for financial support.

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1. Marcano, M. and Barrow, R. F., *Trans. Far. Soc.*, 1970, 66, 2936.
2. Gaydon, A. G., *Dissociation Energies and Spectra of Diatomic Molecules*, Chapman and Hall, London, 1968.

Chenopodium murale: A Local Lesion Host for Cucumber Mosaic Virus (CMV) Isolated from *Vinca rosea*

Chenopodium murale has been reported as a local, lesion host for some plant viroses (Horvath,[1], 1969; Smith[4], 1972; Padma and Summanwar[2], 1973).

Experiments were carried out to determine the utility of *C. murale* as a local lesion host for CMV (spherical) isolated from *V. rosea* (Rajyalakshmi Rao[3], 1976) in addition to *Chenopodium amaranticolor*. The CMV culture was maintained on *Nicotiana tabacum* cv. White Burley and *N. glutinosa* in an insectproof glass-house.

The test plants of *C. murale* were raised in 6 cm pots filled with sterilized soil and kept inside the insectproof glass-house. For inoculations the conventional method of macerating the infected leaf material in pestle and mortar and rubbing the leaves of test plants by means of cotton swab was employed. Within 5–6 days of inoculation, distinct countable 20—22 chlorotic circular lesions were produced by the virus which turned necrotic with a pale halo over a period of 10–12 days. Back inoculation from lesions on *C. murale* produced typical lesions and infected *N. tabacum* and *N. glutinosa* systematically.

The authors are thankful to Dr. V. V. Chenulu, Head of Plant Pathology, I.A.R.I., New Delhi for providing necessary facilities and Dr. V. S. Verma for going through the manuscript and his valuable suggestions.

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1. Horvath, J., *Acta Phytopathologia Acad. Sci. Hungaricae*, 1969, **4**, 337.
2. Padma, R. and Summanwar, A. S., *Curr. Sci.*, 1973, **42**, 620.
3. Rajyalakshmi Rao, D., *Ph.D. Thesis*, B.H.U., Varanasi, 1976.
4. Smith, K. M., *A Text Book of Plant Virus Diseases*, III edn. Longman Group Ltd., London, 1972.

Two New Leaf Spot Diseases

During the year 1972–73 the authors observed two leaf spot diseases, caused by pathogenic fungi in the Botanical Garden, B.H.U. Two pathogens were isolated from infected leaves using Czapek's agar medium at 25 ± 1° C. The cultures of the fungi were identified and deposited in the Department of Botany, B.H.U. The morphological characters of the species were similar to those of type species. The diseased specimens were deposited in Herb Cryptogammae Indicae Orientalis at Division of Mycology and Plant Pathology, I.A.R.I., New Delhi–12. The accession numbers assigned to these are H.C.I.O. 32409 and 32410 for *Terminalia chebula* and *Acorus calamus* hosts respectively. The symptoms of the diseases are described below:

Phoma arcuata (Cooke) Sacc.[1] produced brown bionaceous, irregular, 0·5–5 mm spots on the leaves of *Terminalia chebula* Retz. The colonies on the Czapek's medium showed pycnidia of umber coloured in early stage, later turned black-brown to black, csliotate, and erumpent. Conidia were 1-celled, 6·14 × 4–55.

The infected leaves of *Acorus calamus* Linn. by *Gloeosporium polymorphum* Trin.[2] were showing elongated, rusty pustules. The colonies on Czapek's agar was floccose, cinnemon coloured, erumpent. Conidia were hyaline 1-celled, 8–22 × 3·4 μ.

The pathogenicity tests on their respective hosts were found to be satisfactory. The above-mentioned diseases are now records.

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1. Saccardo, P. A., *Sylloge Fungorum*, 1884, **3**, 100.
2. —, *Ibid*, 1913, **22** (2), 1190.

Occurrence of Barking Deer, *Muntiacus muntijak* Zimmermann from the Prehistoric Site of Kalibangan, Rajasthan

The animal remains excavated by the Archaeological Survey of India in the year 1963–65 from the prehistoric site of Kalibangan in Ganganagar District of Rajasthan, India, contains two skeletal remains of the barking deer, *Muntiacus muntijak* Zimmermann, 1780 (Cervidae: Muntiacinae). These are fragments of the distal end of right femur and distal end of right humerus bearing legend "Site-KLB–2, locus XA 17 qd–4, stratum–2". The maximum length of the distal condylar portion of the right humerus of Kalibangan specimen is 25·8 mm (*cf.* 27·0 mm in a recent specimen in Z.S.I. coll.).

The find of the remains of the barking deer has significance in throwing light on the ancient distribution of this deer in India. The only other record of recovery of barking deer from prehistoric site is a molar tooth from Nagarjunakonda (*ca.* 200–1200 A.D.) in Guntur District (Andhra Pradesh) (Nath[2], 1968).

The present distribution of barking deer in India, according to bibliographic sources, includes Bhutan Duars and Assam, Peninsular India, Ceylon, Burma etc. It does not seem to have been recorded from Rajasthan, and the known habits of this animal preclude its presence in dry arid tracts without floral canopy. In the context of the present distribution of this species in India, the find of fragmentary remains of limb bones of the barking deer in Rajasthan has considerable historical interest, indicating that three millennia ago this tract, now a sandy waste, was well-forested and had the right ecological conditions to support the existence of barking deers. With desertification, the area seems to have become unsuitable for this species which, consequently, seems to have disappeared from there.

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1. Ellerman, J. R. and Morrison-Scott, T. C. S., *Checklist of Palaeartic and Indian Mammals* (1758 to 1946), London, 1951, p. 355.
2. Nath, B., "Advances in the study of prehistoric and ancient animal remains in India". A review Rec. *Zool. Surv. India*, Delhi, 1968, 61 (1 and 2), 58.

A New Fruit Rot of Mango

An undescribed fruit rot disease of mango (*Mangifera india* L.) was observed by the authors and the causal organism was identified as *Myrothecium roridum* (Tode) ex. Fr. (No. IMI 141876). It was observed that slight injury was necessary for onset of infection.

The disease was observed in "Deshi" (local) mango fruit. In the early stage one or two small nearly circular spots appeared on the lateral side of fruit, which were light brown in the centre surrounded by a dark brown band. The decay was confined to the skin of the fruit in the earlier stage, while in the later stages it penetrated the deep mesocarp. Internal tissues appeared dark brown when the fruit was cut open through the infected region. The dark green sporodochia appeared on the infected areas as the disease progressed.

M. roridum elicited good deal of attention from the pathologists as it causes fruit rots and leaf spots of several economically important plants in this country[1,2]. The perusal of literature[3,4] revealed that this pathogen has so far not been reported on mango fruits.

The authors are thankful to the Director, C.M.I., Kew, England, for confirming the identity of the pathogen and to the Department of Atomic Energy, Government of India, for award of Junior Research Fellowship to S. Kumar.

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1. Ponnappa, K. M., *Hyacinth Control Jour.*, 1970, **8**, 18.
2. Ullasa, B. A., Maholay, M. N. and Sohi, H. S., *Curr. Sci.*, 1976, **45**, 601.
3. Srivastava, M. P., Tandon, R. N., Bhargava, S. N. and Ghosh, A. K., *Proc. Nat. Acad. Sci. India*, 1965, **35**, 69.
4. Laxminarayana, P. and Reddy, S. M., *Indian Phytopath.*, 1975, 28, 529.

REVIEWS AND NOTICES OF BOOKS

Far Infrared Astronomy. Edited by Michael Rowan-Robinson. (Pergamon Press, Oxford, England), Pp. i-xiv + 335. Price $ 20.

The book under review is the proceedings of the first International Conference held at Windsor, U.K., during July 1975 and devoted to the whole of field of far infrared astronomy. It is divided into six parts,

The first part, Instrumentation and Atmospheric Constraints, gives several papers describing the techniques that are being used in balloon-borne telescopes for infrared and far infrared observations. One paper deals with the constraints imposed by atmospheric fluctuations on far IR observations even at high altitude sites.

The second part, Solar and Jovian Atmospheres describes observations of the Sun and Jupiter at mm sub-mm and far infrared wavelengths. It is striking to note that very little work has been done on the solar system at these wavelengths. The third part Cosmic Microwave Background, contains several papers on instrumentation and some on observations of the cosmic microwave background below 3 mm wavelength where one expects the turnover in the spectrum. It brings out clearly the severe problems encountered in such measurements like emission from residual earth's atmosphere even at balloon altitudes.

Line Astronomy is discussed in the fourth part which deals mostly with molecular line H_2CO, H and CO) observations at cm and mm-wavelengths in the direction of JR sources. IR and far IR line astronomy is still in its infancy and one hopes this field develops soon. The fifth part, Continuum Emission, contains several papers on measurement and interpretation of continuum emission from galactic and extragalactic objects at mm and IR wavelengths. The last part, Theoretical Models, is devoted to papers giving the theory of IR emission from dust and grains in the interstellar medium.

The book is extremely useful and contains contributions from the stalwarts in the field. The stimulating discussions which followed the presentation of every paper have been recorded meticulously. After going through this book, one is reminded of the Paris Symposium on Radio Astronomy in 1958. One cannot but agree with Dr. Rowan-Robinson, editor of the volume, who hopes "......this collection of papers will provide a stimulating introduction to this rapidly growing field, which stands roughly where X-ray astronomy stood 5 years ago and where radio astronomy did 25 years ago". Let us also hope, as Professor J. Bastin remarks in the introduction, that as the number of far infrared astronomers grow, it may be hoped that at least some may decide to specialise in observations within our own solar system.

This book will be a valuable addition to those libraries which are interested in astronomy and is a must for those who plan to enter the field of IR and far IR astronomy.

The printing and get-up of the book is excellent but one would have liked the paper slightly less glossy to avoid glare as well as stickiness in moist conditions.

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Ammonia (Part III). Edited by A. V. Slack, and G. Russell James. (Marcel Dekker, 270 Madison Avenue, New York 10016), 1977. Pp. xv + 459. Price $ 69-75.

The present book *Ammonia* edited by Slack and James is part 3 of a four volume work on technology and economics of ammonia production. The present volume mainly deals with production of ammonia from synthesis gas. The selection of subjects for various chapters is judicious some of them have been dealt for the first time.

One of the interesting subjects discussed is the centrifugal compressors and their drives the former is more complete than the latter. The case histories of some of the failures of compressors and turbines given will be helpful to ammonia plant operators and designers.

The chapter on ammonia synthesis are quite exhaustive, and these will be helpful to students as well as professional engineers. There seems to be a slight bias for low pressure ammonia synthesis. It should be borne in mind that processes applying higher pressures which are on the market are competitive.

The chapter on commercial ammonia processes will be useful to operators and planners. Listing of number of plants, operating under each of the processes is a good idea. The chapter on the materials of construction deals with reformation and CO_2 removal sections only. Inclusion of other sections would have been quite useful. Use of computers for process analysis is a valuable new feature in this book and will be of interest to Indian Engineers.

The chapter on ammonia production economics tries to confine a vast subject in a single chapter. The section on economy of scale *vs.* market is worth special study by planners. In passing, it should be noted that cost figures given seem to be those valid for pre-energy crisis period.

This book along with its earlier two parts should find a place in the libraries of all technical institutions and fertilizer plant owners. The final and fourth volume of *Ammonia* dealing with storage, handling and transportation of ammonia will be awaited with interest.

H. Srikant.

Biochemical Systems Analysis: A Study of Function and Design in Molecular Biology. By Michael A. Savageau. (Addison Wesley Publishing Company, Mass.), 1976 Pp. 379. Price $ 26.50.

This book is concerned with the analysis of the dynamic properties of interacting biochemical pathways and mathematical description of chemical changes n biological systems. It is divided into three parts. Part I is a kind of introduction in which the nature of biochemical systems, thermodynamics and classical enzyme kinetics are dealt with. Part II deals with more advanced mathematical techniques which include linear and non-linear systems analysis as well as dynamic solutions by computer. Part III is on the application of the mathematical techniques to the understanding of genetic and enzyme regulation in biological systems. The author discusses the difficulties involved in describing complex biochemical changes in mathematical terms and emphasises the need for applying approximations in deriving mathematical equations.

Throughout the book the main topics of discussion are different types of metabolic pathways and their regulation. Attempt is made to give theoretical justification for the observed phenomena. Biochemical pathways take a variety of forms they may be linear, branched, convergent or cyclic. The pathway length, the characteristics of intermediates and the kinetic parameters of the reactions, the strength of feedback inhibitions, the type of regulation etc., are important for the stability of the system. The mathematical analysis presented in the book shows that shorter pathways tend to promote stability of control by feedback inhibition, in addition to conservation of energy. In agreement with this, is the observation that in *E. coli* 12 out of 20 amino acids are synthesized by pathways shorter than 4. Nevertheless in micro-organisms, there are several pathways of 5 to 10 reactions. A case in point is the histidine biosynthetic pathway of *Salmonella typhimurium.* Attempts have have been made to seek alternate explanations in such cases. The stability of histidine biosynthetic pathway has been attributed to the wide range of K_M values of the enzymes of the pathway. Mathematical treatment of the kinetic parameters has shown that such systems indeed can exist.

The book has an author index and a subject index and at the end of each chapter a number of useful references are given. A good background in mathematics and calculus is essential to grasp the contents of the book.

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The Propagation of Tropical Fruit Trees. By Garner, R. J. and Saeed Ahmed Chaudhri. (Commonwealth Agricultural Bureau, Farnham Royal, Slough, SL2 3BN, England), 1976. Pp. xv + 566. Price £ 8.00; Overseas £ 9.60.

This is a publication sponsored jointly by the F.A.O. and the Commonwealth Agricultural Bureau. The publication of this book fills a long void in the compilation of available information on the propagation of tropical fruit trees. Though lot of information is now available on all aspects of propagation of tropical fruits, these remain more or less scattered in different journals, only a few of these are accessible to most of the horticulturists. It is therefore a most welcome development in tropical horticulture that the authors have assigned to themselves.

This comprehensive review deals with the various aspects of propagation. These also include the management of a nursery, preparation of potting composts and even the locally available material that could come very handy in nursery management. In Part I, full details of propagation by cuttings, the planting environment, the source of cuttings and different preplanting treatments for the successful rooting of cuttings are given. In this part is also included, the different methods of grafting, mentioning their advantages and disadvantages. The methods adopted for top working are also mentioned and these are particularly useful in tropical countries where one observes vast areas under seedling trees with good varieties of known performance.

In Part II, the available information on the propagation of individual fruit trees have been mentioned in detail. General information regarding geographical distribution, growth, habit, varieties and diseases and pests have also been provided for each fruit crop which is very valuable. The authors have included almost all the important tropical fruits. However, it would have been comprehensive if the propagation methods employed in citrus, banana and pineapple were also included in this book.

Number of useful and clear diagrams have been given in the book which adds to its value.

To summarise, the book is extremely useful to horticultural scientists, nurserymen and to amateur horticulturist and should find its place in every library dealing with horticulture.

G. S. RANDHAWA.

ALL INDIA SEMINAR ON ICHTHYOLOGY

The first 'All India Seminar on Ichthyology (AISI)' was held recently from May 23 to 26, 1977 at Zoology Research Laboratories. M.M. College, Modinagar, under the auspices of University Grants Commission and the leadership of Dr. O. P. Saxena, The seminar was inaugurated by Hon'ble Dr. P. C. Chunder, Union Minister for Education. In all, 74 Ichthyologists from different parts of the country took part in the symposium. The business session of the seminar began from 24th May and these included sessions on (1) Morphology and Anatomy, (2) Physiology and Biochemistry, (3) Cytology and Histochemistry, (4) Fishery Biology and (5) Systematics, Zoogeography, Ecology and Pollution.

The seminar was concluded on 26th May 1977 under the presidentship of a veteran Ichthyologist Prof. V. P. Agrawal, Principal, D. A. V. College, Muzaffarnagar and the delegates unanimously resolved that the first 'All India Seminar on Ichthology' was a long-felt desire of the Ichthyologists and strongly recommended that similar seminars on Ichthyology should be organised frequently in different parts of the country.

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TORSIONAL POTENTIALS FROM OBSERVED DIPEPTIDE CONFORMATIONS IN PROTEIN STRUCTURE DATA

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ABSTRACT

The (ϕ, ψ)-values obtained from the crystal structure data of twentytwo globular proteins have been used to obtain the less understood torsional potential functions $V_t(\phi)$ and $V_t(\psi)$, for rotations around the single bonds N—C$^{\alpha}$ and C$^{\alpha}$—C respectively, in polypeptides. The method developed to obtain these functions, from an analysis of the crystal structure data and energy calculations on dipeptide units having different side chains, is discussed very briefly. The newly obtained torsional potential functions are: $V_t(\phi) = -1{\cdot}0 \cos(\phi + 60°)$ and $V_t(\psi) = -0{\cdot}5 \cos(\psi + 60°) - 1{\cdot}0 \cos(2\psi + 30°) - 0{\cdot}5 \cos(3\psi + 30°)$. The (ϕ, ψ)-Ramachandran energy map obtained for an 'averaged' dipeptide unit, using these functions, is found to be in very good agreement with the energy map obtained from the experimental protein data.

INTRODUCTION

THE prediction of preferred conformations of polypeptide chains, obtained by using semiempirical potential energy functions, depend on the accuracy of these functions. Ramachandran[1] has indicated that the potential functions, which are commonly used to predict the conformation of biopolymers, must be tested using data obtained from various physico-chemical methods on related small molecules and macromolecules. In this connection, from the studies on the packing of molecules in the crystalline state, it was shown that the nonbonded potential energy functions used in this laboratory, as well as the functions used by various other groups for the study of biopolymer conformation, give data which are in good agreement with experimental results[2, 3]. However, studies carried out in our laboratory on model compounds using semi-empirical quantum chemical methods have indicated that the torsional potential function $V_t(\phi)$, for rotations ϕ around the single bond N—C$^{\alpha}$, is small[4], while the torsional potential function $V_t(\psi)$, for rotation around the C$^{\alpha}$—C single bond is mainly two-fold in nature, with a large barrier of the order of 4·0 kcal/mole[5]. From these studies the function $V_t(\psi)$ was suggested to be of the form

$$V_t(\psi) = \tfrac{1}{2} V_{\psi_0} (1 - \cos 2\psi)$$

with

$$V_{\psi_0} = 4{\cdot}0 \text{ kcal/mole.} \qquad (1)$$

This is different from the previously accepted functions both in the form of $V_t(\psi)$ and the value of its barrier. Thus there is a need for a further investigation in order to obtain the correct nature of these less understood potential energy functions, $V_t(\phi)$, and $V_t(\psi)$, for peptide units. Therefore, we have analysed the observed distribution of the quantities (ϕ, ψ) in the crystal structure data of twentytwo globular proteins and derived from them the function $V_t(\phi)$ and $V_t(\psi)$. The method developed for this purpose is discussed briefly in the next section, and the results are outlined in the succeeding section.

METHOD

In the semi-empirical conformational energy calculations on polypeptides and proteins, the total potential energy is usually partitioned as

$$V = V_{tot} = V_{nb} + V_{es} + V_t(\phi) + V_t(\psi) + V_{hb} + V_{ss} \qquad (2)$$

where, V_{nb} is the nonbonded interaction energy, V_{es} the electrostatic interaction energy, $V_t(\phi)$ and $V_t(\psi)$ are torsional potential energy functions, V_{hb} is the hydrogen bond energy and V_{ss} is the solute-solvent interaction energy. Out of these, the last term is least understood and very often it is neglected. We shall also do so. Similarly, if only short range interactions are considered, which form the dominant factor determining the conformation in globular proteins[6], then V_{hb} also can be neglected. Thus, the total potential energy can be written in the form

$$V(\phi, \psi) = V_1(\phi, \psi) + V_2(\phi, \psi) \qquad (3)$$

where

$$V_1 = V_{nb} + V_{es} \qquad (4)$$

and this term is dependent on the nonbonded interatomic distances r_{ij}, and

$$V_2(\phi, \psi) = V_{tor}(\phi, \psi) = V_t(\phi) + V_t(\psi) \qquad (5)$$

and this term is dependent only on the values of the dihedral angles (ϕ, ψ). Of these, the total potential energy in equation (3), namely $V(\phi, \psi)$, can be determined for each amino acid residue from the observed distribution $P(\phi, \psi)$, in the (ϕ, ψ)-plane, as obtained from the (ϕ, ψ)-data in a large number of proteins, using the Boltzmann relation[7, 8]:

$$P(\phi, \psi) \propto \exp - V(\phi, \psi)/RT. \qquad (6)$$

On the other hand, $V_1(\phi, \psi)$ can be calculated, for each amino acid residue, using the known potential energy functions V_{nb} and V_{es} for which the parameters employed are well established. We have used, for, this purpose, the 6-exp nonbonded interaction potential function with the constants given by Chandrasekaran and Balasubramanian[9], except for those involving hydrogens, for which a softened potential is used, by reducing the van der Waals radius of hydrogen by 0·1 Å[10, 11]. The electrostatic energy V_{es} was calculated using the monopole approximation and an effective dielectric constant[12].

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Knowing V and V_1, the effective torsional potential energy, $V_2(\phi, \psi)$, was deduced using eq. (3). This was then analysed as the sum of two components $V_t(\phi)$ and $V_t(\psi)$, as in eq. (4) and each was represented as a sum of Fourier components of the form

$$V_t(\theta) = V_{\theta 1}\cos(\theta - \delta_{\theta 1}) + V_{\theta 2}\cos(2\theta - \delta_{\theta 2}) + V_{\theta 3}\cos(3\theta - \delta_{\theta 3}) \qquad (7)$$

in which $V_t(\phi)$ and $V_t(\psi)$ are obtained by substituting for θ, ϕ and ψ respectively.

To start with, $V_t(\phi)$ was put equal to zero, as indicated by Kolaskar *et al.*[4] and the experimental data for $V_{exp}(\psi)$ were obtained by summing the experimental data of $P_{exp}(\phi, \psi)$ over the variable ϕ and thus obtaining $P_{exp}(\psi)$, andthen using a formula similar to eq. (6) to convert $P_{exp}(\psi)$ into $V_{exp}(\psi)$. Similarly, $V_1(\psi)$ values were obtained from theoretical studies on dipeptide units while $V_t(\psi)$ values were got using eq. (3). The Fourier coefficients $V_{\psi 1}$, $V_{\psi 2}$ and $V_{\psi 3}$ as well as the phase angles, $\delta_{\psi 1}$, $\delta_{\psi 2}$ and $\delta_{\psi 3}$, were obtained from these data of $V_t(\psi)$ *versus* ψ by standard procedures. These values were refined by calculating back

$$V_{th}(\psi) = V_1(\psi) + \sum_{k=1}^{3} V_{\psi k}\cos(k\psi - \delta_{\psi k}) \qquad (8)$$

and converting these into theoretical probability data *viz.*, $P_{th}(\psi)$. Then, $V_{\psi k}$ and $\delta_{\psi k}$ were refined by finding the best fit between $P_{th}(\psi)$ and $P_{exp}(\psi)$, as judged by the value of the R-factor

$$R = \frac{\Sigma \left| P_{th} - P_{exp} \right|}{\Sigma P_{exp}} \qquad (9)$$

Having thus refined the ψ-potential, this was used to obtain $P_{th}(\phi)$ from $V_{th}(\phi, \psi)$ and $V_t(\phi)$, in exactly the same manner as was done for the ψ-potential. Then $V_{\phi k}$ and $\delta_{\phi k}$ ($k = 1, 2, 3$) were determined, first roughtly by Fourier analysis, and then refined by making use of the R-value. This completes one cycle of refinement.

In the same way, three cycles of refinement were carried out, first by summing over ϕ and analysing $V_t(\psi)$, and then by summing over ψ and analysing $V_t(\phi)$, each time. The accuracy attainable for $V_{\phi k}$ and $V_{\psi k}$ was only of the order of 0·5 kcal/mole and for $\delta_{\phi k}$ and $\delta_{\psi k}$ was the order 30°.

RESULTS AND DISCUSSION

The functions, $V_t(\phi)$ and $V_t(\psi)$, thus obtai after three cycles of refinement for a dipeptide containing an Ala side chain, are

$$V_t(\phi) = -1·0\cos(\phi + 60°) - 0·5\cos \times (2\phi + 30°)$$

and

$$V_t(\psi) = -0·5\cos(\psi + 60°) - 1·0\cos \times (2\psi + 30°) - 0·5\cos(3\psi + 30°).$$

Applying these torsional potential functions, the v of $V_{tot}(\phi, \psi)$ was calculated, without including hy gen bond energy, for an Ala side chain. For co parison with the observed data of $P_{exp}(\phi, \psi)$, calculations were made for three values of τ namely, 105°, 110° and 115°, and the total poten energy was obtained as a "weighted" average these three. The distribution of $V_{th}(\phi, \psi)$ t obained is shown in Fig. 1 by continuous lines. T isoenergy contours are drawn at intervals of 0·5 k mole, above the absolute minimum. In the sa figure, the dotted curves correspond to the isoene contours which were obtained from the values $P_{exp}(\phi, \psi)$, calculated for Ala residues, from the dis bution in the (ϕ, ψ)-plane observed in the crys structure data of the globular pioteins mentior above. It must be noted tht we have consider only those Ala residues which were found to occ

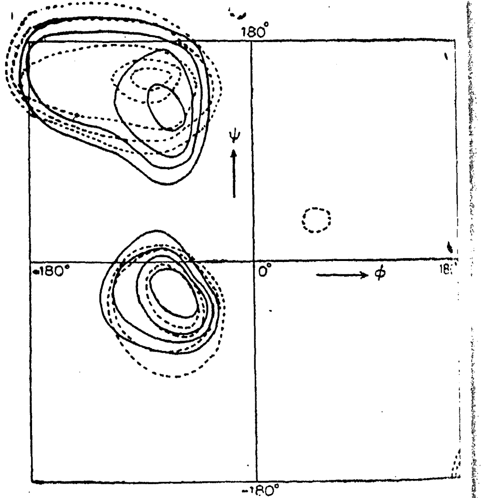

FIG. 1. Isoenergy contours in the (ϕ, ψ)-plane i an alanine dipeptide unit at intervals of 0·5 kcal/mo The continuous curves are drawn using values $V_{tot}(\phi, \psi)$ obained from calculations using the fu tions $V_t(\phi)$ and $V_t(\psi)$, as given by eq. (10) and (11). The dotted curves are drawn by using $P_{exp}(\phi, \psi)$ data of Ala residues from 22 globu proteins.

outside the α-helical region and have avoided the influence of long range interactions to a very good extent. Hence, this map, obtained from the observed dipeptide conformations in protein structure data, can be directly compared with that obtained from calculations on a dipeptide unit having an Ala side chain. A glance at Fig. 1 would indicate the very good agreement between theory and experimental data. However, we would like to caution that the experimental isoenergy curves are approximate as we had to draw them using only data from about 300 examples.

Similar calculations were carried out on dipeptides having Val, Leu, Ile, Ser, and Phe side chains, and the functions $V_t(\phi)$ and $V_t(\psi)$ were obtained in each case making use of the experimental crystal structure data from globular proteins and the calculated values of $V_1(\phi, \psi)$, taking into account the interactions with the side chains and the torsional potential for the side chains.

These studies have given potential functions having only marginal differences in the values of the parameters for the functions $V_t(\phi)$ and $V_t(\psi)$. Considering all these functions obtained for the different residues, a function which fits best the experimental protein data on these residues was deduced. This is given by

$$V_t(\phi) = -1 \cdot 0 \cos(\phi + 60^\circ) \tag{12}$$

and

$$V_t(\psi) = -0 \cdot 5 \cos(\psi + 60^\circ) - 1 \cdot 0 \cos \times (2\psi + 30^\circ) - 0 \cdot 5 \cos(3\psi + 30^\circ) \tag{13}$$

These functions were then used to calculate the total "averaged" potential energy distribution in the (ϕ, ψ)-plane, for the different dipeptide units having side chains Ala, Val, Leu, Ile, Ser and Phe. The potential energy values were properly normalised and multiplied by a statistical weight factor proportional to the number of examples of each type. The potential energy map thus obtained is shown in Fig. 2 (*a*) and its isoenergy contours may be compared with those in Fig. 2 (*b*), which are obtained by using the experimental data $P_{exp}(\phi, \psi)$ for the same six amino acid residues. It can be seen from Fig. 2 that the experimental curves and theoretical "averaged" energy contours agree very well. This amount of agreement has never been obtained earlier between theory and experiment.

It should be mentioned that the potential functions (12) and (13) refer to the usual L-amino acid residues. For a D-residue, the corresponding equations will be similar to (12) and (13), with the same $V_{\phi k}$ and $V_{\psi k}$, but with $\delta_{\phi k}$ and $\delta_{\psi k}$ of the same magnitudes as in (12) and (13), but of opposite signs.

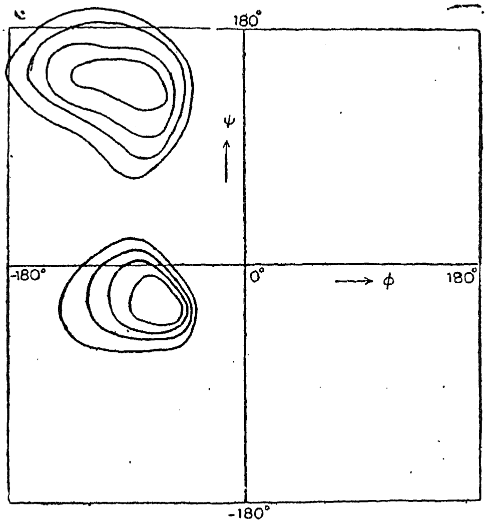

FIG. 2 (*a*). Isoenergy contours of $V_{tot}(\phi, \psi)$ drawn, at intervals of 0·5 kcal/mole, for a dipeptide unit, after properly averaging over different side chains (*see* text). The fnctions $V_t(\phi)$ and $V_t(\psi)$ as given in eq. (12) and eq. (13) were used for the total potential energy calculations.

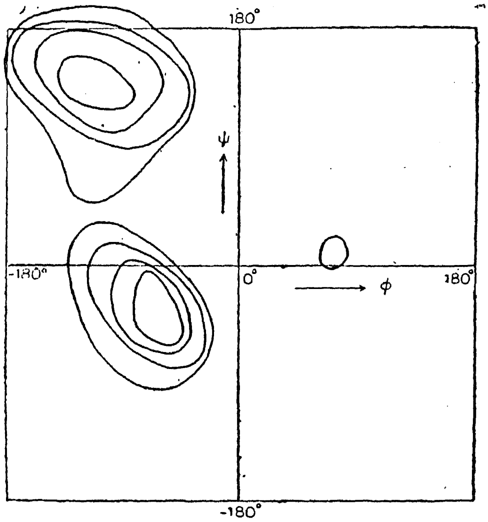

FIG. 2 (*b*). Isoenergy contours, at intervals of 0·5 kcal/mole, drawn using the (ϕ, ψ) data for Ala, Val Leu, Ile, Phe and Ser residues from twentytwo proteins. Note the very good agreement between Fig. 2 (*a*) and Fig. 2 (*b*).

The results presented here clearly indicate that the data on macromolecules, which are accumulating very rapidly, can be used to derive semiempirical potential energy functions, which can be used no only in the study of macromolecules, but also for small molecules. The details of the results presented here are expected to be published elsewhere.

ACKNOWLEDGEMENT

We thank Prof. G. N. Ramachandran for suggesting this problem.

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1. Ramachandran, G. N., In "Conformation of biological molecules and polymers", (Bergman, E. D. and Pullman, B., Eds.), *Israel Acad. of Sci. and Humanities*, Jarusalem, 1973, pp. 1-11.
2. —, Sarathy, K. P. and Kolaskar, A. S., *Z. fur. Kristall.*, 1973, **138**, 299.
3. Kolaskar, A. S., *Ph.D. Thesis*, Indian Institute of Science, Bangalore 1976.
4. —, Sasisekharan, V. and Sarathy, K. P., *Theoret. Chim. Acta (Berl.)*, 1975, **38**, 109.
5. —, Sarathy, K. P. and Sasisekharan, V., *Curr. Sci.*, 1975, **44**, 35.
6. Chou, P. Y. and Fasman, G. D., *Biochemistry*, 1974, **13**, 211.
7. Flory, P. J., *Statistical Mechanics of Chain Molecules*, Wiley-Interscience, New York, 1969.
8. Ramachandran, G. N., Unpublished, 1977.
9. Chandrasekharan, R. and Balasubramanian, R., *Biochem. Biophys. Acta*, 1969, **188**, 1.
10. Ramachandran, G. N., *Jour. Ind. Inst. Sci.*, 1975, **56**, 375.
11. Hagler, A. T. and Lapiccirella, A., *Biopolymers*, 1976, **15**, 1167.
12. Ramachandran, G. N. and Sasisekharan, V., *Advan. Protein Chem.*, 1968, **23**, 283.

ELECTROPHORESIS OF ^{32}P-LABELLED OLIGONUCLEOTIDES ON THIN-LAYER DEAE-CELLULOSE FOR RAPID SEPARATION IN FINGERPRINTING

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THE two-dimensional fractionation procedure for radioactive oligonucleotides, developed by Sanger *et al.*[1] is now widely used in fingerprinting and sequencing of nucleic acids. In this procedure separation in the first dimension is by electrophoresis on a cellulose acetate membrane strip at high voltage and in the second dimension on a DEAE-cellulose paper. Brownlee and Sanger[2] have employed chromatography on thin-layer DEAE-cellulose for fractionation of large oligonucleotides, especially deoxy-oligonucleotides. Grohmann and Sinsheimer[3] have noted certain advantages in carrying out the first dimension electrophoresis on thin-layer cellulose instead of cellulose acetate. We have devised a simple procedure for carrying out electrophoresis in the second dimension on a thin-layer DEAE-cellulose plate. In this method voltages of the order of 50–60 per cm can be applied without appreciable heating effect and the fractionation completed in a few hours.

EXPERIMENTAL

Mycobacterium smegmatis, used in these studies, was from Microbiology and Cell Biology Laboratory of this Institute. DEAE-cellulose (0·85 meq/g) and cellulose powder (microcrystalline) were purchased from Centron Research Laboratory, Bombay. RNase T_1 and RNase A were from Sigma Chemical Company, St. Louis. Carrier free ^{32}P-orthophosphate was from Bhabha Atomic Research Centre, Bombay. All other reagents were of analytical grade.

Preparation of ^{32}P-labelled oligonucleotides

^{32}P-labelled total RNA was isolated from *Mycobacterium smegmatis* grown with radioactive phosphate in low phosphate medium[4,5]. 5S RNA was separated by polyacrylamide gel-electrophoresis and digested with RNase T_1 or RNase A according to the procedure of Sanger and Brownlee[6].

Preparation of thin-layer DEAE-cellulose plates

DEAE-cellulose and microcrystalline cellulose in the required proportions were thoroughly mixed with water (6 ml per g of mixture) in a Waring Blendor and the slurry was applied to glass plates using a Desaga spreader with thickness setting at 0·5 mm. The plates were dried at 60–80° C.

Determination of base composition

The radioactive spots, as revealed by autoradiography, were scraped from the thin layer plate, eluted with 30% triethylammonium carbonate and digested with 0·2 M NaOH. The mononucleotides were separated by electrophoresis at pH 3· on Whatman No. 1 paper using cold nucleotides as markers, and counted in a liquid scintillation counter

PROCEDURE

RNase T_1 or RNase A digest of ^{32}P-labelled 5S RNA was subjected to electrophoresis in the first dimension on a cellulose acetate membrane strip and the oligonucleotides were transferred to the thin-layer DEAE-cellulose plate as described by Brownlee and Sanger[2]. After washing with alcohol the plate was sprayed with a buffer, consisting of pyridine (0·5%), acetic acid (5%) and EDTA (0·005 M), the pH being 1·9. A wet Whatman No. 1 paper was attached to that end of the plate where oligonucleotides were transferred from the cellulose acetate paper. The plate was placed in the tank (Fig. 1) and subjected to electrophoresis.

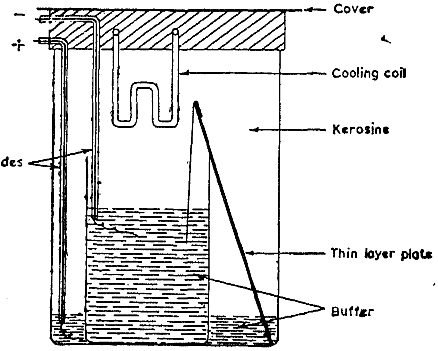

FIG. 1. Electrophoresis apparatus. The chromatographic tanks used were of dimension 25 × 30 × 40 cm and 10 × 25 × 25 cm. The buffer containing a mixture of 2·5% formic acid, 8·7% acetic acid and 0·005 M EDTA (pH 1·9) was added to a height of 3 cm in the larger tank and 16 cm in the smaller tank. Kerosene was added to fill the larger tank. Tap water was circulated though the cooling coils.

The apparatus was made out of two chromatographic tanks of unequal dimensions. The smaller tank formed the cathode compartment and the larger one the anode compartment. The space above the buffer in the two tanks was filled with kerosene which acted as the coolant. A wooden frame fixed to the top of the larger tank served as a convenient support for the electrodes and the cooling coils. The electrodes were of platinum wire. The smaller tank acted as a support for keeping the thin layer plate in a slightly slanting position and the Whatman No. 1 paper conducted the current.

RESULTS AND DISCUSSION

With the setup described above a voltage of 50–60 per cm could be applied without appreciable heating effect and fractionation completed in 1 to 2 hr. Good resolution of the oligonucleotides, as revealed by autoradiography, was observed. A typical fingerprint of 5S RNA of *Mycobacterium smegmatis* is shown in Fig. 2. The radioactive spots were scraped out from the plate and they were analysed for their base composition as described under experimental. The base compositions of the fragments obtained by digestion with RNase T_1 are shown in Table I. These results were highly reproducible. Some of the faint spots also were analysed. Two spots thus analysed were (CA)G and (UC)G (not shown in Table I). Movement of the various oligonucleotides on thin layer was somewhat similar to that observed by Sanger and Brownlee on DEAE-cellulose paper[6]. For example, the relative positions of spots 1, 2, 3, 6 and 11 in Fig. 2 which represent G_p, cyclic G_p, C_pG_p, $C_pC_pG_p$ and $C_pG_pC_pG_p$ are identical to those on DEAE-cellulose paper. Similar regularities with respect to other oligonucleotides were observed both in RNase T_1 (Fig. 2) and RNase A digests (not shown). Spots 9 and 10 in the RNase T_1 digest had the same base composition. It is evident that one of them must have the sequence AUG and the other UAG. Such separation of isomers have been observed on DEAE-cellulose paper[6]. Being smaller, fingerprints of *t*RNAs, in general, can be expected to be simpler and the separation of oligonucleotides better than that for 5S RNA shown in Fig. 2.

TABLE I

Base composition of RNase T_1 fragments of Mycobacterium smegmatis

Spot No.	Base Composition	Spot No.	Base Composition
1	G	13	(C_2U) G
2	G ! (cyclic)	14	(CA_2) G
3	GC	15	AAAG
4	AG	16	(CAU) G
5	UG	17	(CAU_2) G
6	CCG	18	(C_3A_2) G
7	(AC) G	19	$(C_2A_2U_2)$ G
8	AAG	20	(C_5AU_2) G
9	(AU) G	21	$(C_4A_3U_2)$ G
10	(AU) G	22	$(C_4A_2U_3)$ G
11	CCCG	23	pGp
12	(C_2A) G	24	A_2C

The radioactive spots were analysed as described under Experimental. The ratio of bases is expressed relative to the counts for G. The sequences of the nucleotides within brackets are not known.

Separation was better when nucleotides moved down the plate than when they moved up.

In other words, for good resolution oligonucleotides must be applied on the top of the plate (*see* Fig. 1). Time for electrophoresis depended to some extent on the relative proportion of DEAE-cellulose in the thin-layer mixture; lower amounts of DEAE-cellulose in the mixture decreased the time for separation. Optimum resolution was, however, obtained with DEAE-cellulose to cellulose powder in the ratio 1 : 2, in about 2 hr. It usually takes about 16 hr for fractionation on DEAE-cellulose paper. Comparison of the base composition of various oligonucleotides showed that the 5S RNA of *Mycobacterium smegmatis* was different from that of other bacteria[7].

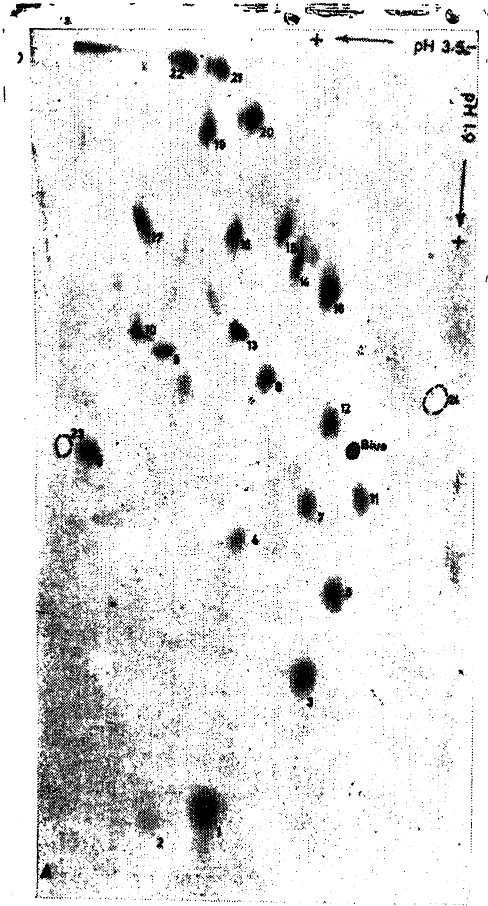

FIG. 2. Autoradiograph of RNase T_1 digestion products of 5S RNA. Thin layer DEAE-cellulose was prepared by coating a 20 × 40 cm glass plate with a mixture of 2 g of DEAE-cellulose and 4 g of microcrystalline cellulose blended with 36 ml of distilled water. Electrophoresis was carried as described in the section on experimental. A voltage of 4,000 for 2 hr was applied. Current gradually decreased from 35 mA to 20 mA.

The method has certain obvious advantages. As the time for the second dimension separation is only 1–2 hr, electrophoresis in both directions can be completed on the same day and fingerprinting time reduced considerably. The saving in time is advantageous when dealing with ^{32}P label which has a short half life. The ratio of DEAE-cellulose to cellulose powder may be varied to get an altered resolution if desired. Larger fragments are expected to separate better by decreasing the proportion of DEAE-cellulose in the thin layer mixture. This method is thus more advantageous in fingerprinting high molecular weight RNAs. It is more convenient to handle DEAE-cellulose plates than DEAE-cellulose paper as the latter is very fragile. Besides the apparatus can be easily assembled in any laboratory.

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1. Sanger, F., Brownlee, G. G. and Barrell, B. G., *J. Mol. Biol.*, 1965, 13, 373.
2. Brownlee, G. G. and Sanger, F., *Eur. J. Biochem.*, 1969, 11, 395.
3. Grohmann, K. and Sinsheimer, R. L., *Anal. Biochem.*, 1975, 64, 214.
4. Brownlee, G. G. and Sanger, F., *J. Mol. Biol.*, 1967, 23, 337.
5. DuBuy, B. and Weissman, S. M., *J. Biol. Chem.*, 1971, 246, 747.
6. Sanger, F. and Brownlee, G. G., In : L. Grosman and K. Moldave (Editors), *Methods in Enzymology*, Vol. XII A, Academic Press, New York, 1961, p. 361.
7. Barrell, B. G. and Clark, B. F. C., *Handbook of Nucleic Acid Sequences*, Joynson Bruvvers, Oxford, 1974, p. 68.

GLYCEROL VARIATION IN *PHILOSAMIA RICINI* HAEMOLYMPH DURING LARVAL-PUPAL DEVELOPMENT

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ABSTRACT

Studies on glycerol concentration in the haemolymph of *P. ricini* during larval-pupal development reveals that the initial low level during the fourth instar stage increases gradually with growth and the highest glycerol accumulation occurs in the fifth instar larva. On the onset of metamorphosis, after a significant depletion, once again glycerol accumulates during puparium-pupa transformation whereafter till ecdysis a U-shaped pattern is observed.

INTRODUCTION

GLYCEROL an important component of all glycerides that constitute the neutral fraction of lipids has been reported by Wyatt and Meyer[1] to occur in the free form in the pupal haemolymph of the silk moth *Hyalophora cecropia.* As the pupal development proceeds there is a gradual accumulation but when the diapause is broken and adult development commences, glycerol rapidly disappears. This finding of Wyatt and his co-workers[1-3] was extended to other species of insects and subsequently confirmed[4,5]. Independently Chino[6,7] reported the occurrence, not only of glycerol but also of sorbitol in *Bombyx mori* embryo during diapause. Curiously however, the presence of glycerol was not detected in the larval blood of all the insects investigated.

Detection of glycerol during paper partition chromatography of larval haemolymph of *Philosamia ricini,* the Eri silk worm, prompted the study of its variation in the haemolymph of this economically important insect during larval-pupal development.

MATERIALS AND METHODS

Larvae of *P. ricini* were reared in the laboratory as described earlier[8].

Haemolymph was collected and pooled in three lots, each from 12 chilled larvae picked at random from the colony during different developmental stages. A small incision was made on the dorsal side of the insect with a sharp stainless steel blade and the pale yellow coloured fluid was allowed to ooze in a centrifuge tube kept immersed in crushed ice and containing a few crystals of thiourea to prevent darkening of the haemolymph.

To obtain pupal haemolymph, an incision was made on the anterior end of the dorsal side of the pupa with a sharp pair of scissors and by applying gentle pressure, the fluid was collected in a pyrex container kept cooled in freezing mixture.

Presence of glycerol in the larval and pupal haemolymph was detected by paper partition chromatography employing a mixture of butanol-acetic acid-water (5 : 1 :2, v/v/v) as irrigating solvent. Glycerol was visualized on the dried chromatogram as described by Hough[9] and Evans and Dethier[10] by spraying it first with sodium periodate solution (0·5% w/v) followed by freshly prepared silver nitrate [5%, w/v, –ammonia (sp. gr. 0·8, 5 : 1, v/v)] mixture after drying the chromatogram. Appearance of dark spots having the same R*f* as that of the authentic reference spot of glycerol along with the test solution on the same chromatogram in three different solvent systems cosfirmed the presence of glycerol.

The chromatogram on being left overnight, developed another spot having a lower R*f* than the reference sorbitol. This compound is under investigation.

Glycerol was estimated by the method of Lambert and Neish[11] as modified by Korn[12]. A suitable volume of haemolymph was deproteinized by the addition of an equal volume of trichloroacetic acid (10%, w/v) and after allowing to stand in the cold for 2–3 min. was centrifuged at 3000 rpm for 10 min. Aliquots 0·5–1 ml) from the supernatant in triplicates were employed for glycerol assay.

RESULTS AND DISCUSSION

With an initial high concentration after ecdysis to the fourth larval instar stage (Fig. 1), glycerol appears to have been utilized as evinced by its significant depletion in the two-day old larva. This is subsequently, followed by a period of accumulation all through fourth instar stage and culminating maximally on the eve of pupation. However, immediately after pupation, glycerol content gets depleted in the zero day pupa where it stands at the lowest level ever recorded during larval development.

During metamorphosis glycerol accumulates at the time of the transformation of the puparium to pupa on days 3/4 whereafter till emergence, it traces more or less a U-shaped pattern with minimal concentration on days 9 and 10. On emergence even (day 15) however, glycerol once again records the same concentration as during puparium-pupa transformation.

Despite the fact that glycerol is a normal metabolic product in animals, its occurrence in appreciably high concentrations in insect blood and tissues at various stages of development is rather extraordinary[4-6]. However, its accumulation was observed exclusively at the stage when the species underwent diapause during adverse conditions of environment in winter.

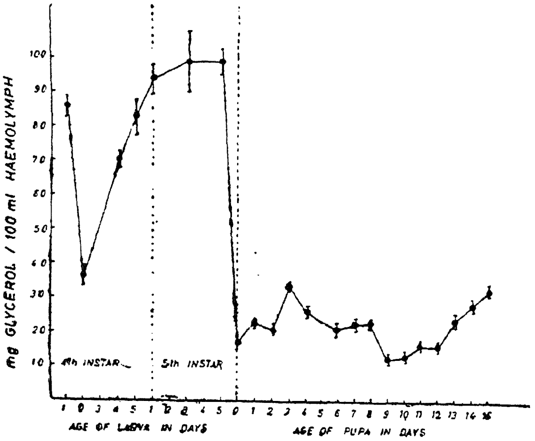

FIG. 1. Variation in haemolymph glycerol concentration during larval-pupal development of *P. ricini.*

Protection from tissue injury due to freezing by the addition of glycerol[13] led to the suggestion and subsequent confirmation[4-5] that glycerol accumulation in insects was a physiological adaptation providing resistance to low temperature. However, Wyatt and Meyer's[1] observations hardly support any such correlation between the two. Likewise, the present investigation in *P. ricini* also does not record any relationship between the presence of glycerol and cryoprotection and what is more *P. ricini* is a non-diapausing lepidopteran.

Following Chino's demonstration[7] of the appearance of glycerol with the simultaneous disappearance of glycogen in *B. mori* tissue, glycogen assays were carried out in the haemolymph of *P. ricini.* However, the results did not afford any supporting data to presume glycogen to be the source of glycerol.

Lepidopteran insects feed voraciously during larval development and consequently exhibit extremely high rate of metabolic activity. Therefore the accumulation of the highest concentration of glycerol during this period suggests high lipolytic and low α-glycerophosphate dehydrogenase activity both contributing to the formation and accumulation of glycerol.

Earlier investigations[14] have revealed that total lipids increase steadily through larval development and attain the maximum at the fifth instar stage. However, on the commencement of spinning, it is followed by a steep fall and then by a rise whereafter an almost constant level is maintained. The variation pattern of lipolytic activity[15] during embryonic and larval-pupal development also further supports the view that the haemolymph glycerol has its origin from lipids.

The lipids that are synthesized in the alimentary tract from the ingested food by a phytophagous insect are transported *via* haemolymph to different tissues. Since neutral lipids are incapable of being transported as such, the intestinal lipase brings about their hydrolysis to mono, di and triglycerides along with the simultaneous release of free glycerol and free fatty acids in the haemolymph. On the other hand, the biosynthesis of neutral lipids from the available constituents occurs in the fat body of the insect. Accordingly, it could be speculated that the fat body withdraws glycerol from the haemolymph as and when required for esterification, suggesting that glycerol variation in larval haemolymph is indicative of lipolysis and lipogenesis occurring in the alimentary tract and the fat body.

Developing adult tissues of *H. cecropia* have been observed to incorporate labelled glycerol at a high rate[16]. Thus the role of glycerol in insects could be considered not only as a cryoprotective agent[17] but also as a precursor of phospholipids and triglycerides.

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1. Wyatt, G. R. and Meyer, W. L., "The Chemistry of insect haemolymph III," *Glycerol, J. gen. Physiol.*, 1959, 42, 1005.
2. — and Kalf, G. F., *Proc. 10th Internat. Congr. Entomol.*, Montreal. 1958, 2, 333.
3. —, Meyer, W. L. and Kropf, R., *Fed. Proc.*, 1958, 17, 340.
4. Salt, R. W., *Can. Ent.*, 1957, 89, 491.
5. —. *Nature*, 1958, 181, 1281.
6. Chino, H., *Ibid.*, 1957, 180, 606.
7. —, *J. Insect Physiol.*, 1959, 2, 1.
8. Pant, R. and Lacy, P. S., *Ind. J. Biochem.*, 1968, 5, 13.
9. Hough, L., *Nature*, 1950, 165, 400.

10. Evans, D. R. and Detheir, V. G., *J. Insect Physiol.*, 1957, **1**, 3.
11. Lambert, M. and Neish, A. C., *Canad. J. Res.*, 1950, **28 B**, 83.
12. Korn, E. D., *J. Biol. Chem.*, 1955, **215**, 1.
13. Lovelock, J. E., *Biochem. J.*, 1954, **56**, 265.
14 Pant, R., Nautiyal, G. C. and Singh, J. B., *Ind. J. Biochem. Biophys.*, 1973, **10**, 116.
15. Pant, R. and Nautiyal, G. C., *Proc. Ind. Acad. Sci.*, 1974, **59**, 230.
16. Habibulla, M. and Gilbert, L. I., Reference cited in *Advances in Insect Physiology*. 1965, **2**.
17. Touster, O. and Shaw, D. R. D., "Biochemistry of the acyclic volyols," *Physiol. Rev.*, 1962, **42**, 181.

EFFECT OF THE SCORPION, *HETEROMETRUS FULVIPES*, VENOM ON GLYCOLYTIC ACTIVITY IN THE BRAIN, MUSCLE AND LIVER OF FROG

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ABSTRACT

The effect of *Heterometrus fulvipes* venom on glycolytic activity was studied in frog brain, muscle and liver during a period of 48 hours. There was a rapid increase in total (*ab*) and active (*a*) phosphorylases in the three tissues both *in vivo* and *in vitro* conditions. The increase was more in liver than muscle and brain. Glycogen content decreased significantly in the three tissues. Lactate dehydrogenase activity also increased uniformly in all the three tissues both *in vivo* and *in vitro*. Lactic acid level increased in liver and decreased in muscle and brain. All these changes occurred progressively reaching maximum around 12–24 hours and then onwards tending towards normal level except in necrotic muscle. These changes suggest sympathetic stimulation by *H. fulvipes* venom and the prevalence of anaerobic condition after venom poisoning.

INTRODUCTION

IT was observed that *H. fulvipes* venom suppresses aerobic enzyme systems and enhances lytic enzyme systems both *in vivo* and *in vitro* conditions in brain, muscle and liver[1,2]. *H. fulvipes* venom was also known to alter metabolic activities[1]. It was reported that *Leiurus quinquestriatus* venom inhibited catalase activity in human erythrocytes[3], while *Buthus minax* venom was found to inhibit succinic dehydrogenase and acetylcholinesterase activity levels[4] in mouse tissues. It was reported that scorpion venom produces hyperglycemia[5,6]. It was also known that the effect of venom varies with the scorpion species and the target tissue[7]. Hence the present study reports the *in vivo* and *in vitro* effect of the venom of the less virulent type of the scorpion (chactoid scorpion), *H. fulvipes*, venom on the glycolytic activity in brain, muscle and liver of frog, *Rana hexadactyla*, which was found to be relatively resistant to venom action[1].

MATERIALS AND METHODS

Venom was collected from the scorpions by applying electrical stimulation to the postabdominal region of the animal and was collected into microbeakers kept at ice-cold temperature. Protein content of the venom, estimated by Lowry *et al.* method[8], was taken as criterion to express the venom quantity. Glycogen[9] and lactic acid[10] contents were estimated in normal and experimental tissues. Phosphorylase (*a* and *ab*) was assayed in the direction of glycogen synthesis[11]. The enzyme activity was expressed as μ moles of Pi formed/mg protein/hour. Lactate dehydrogenase activity was assayed using lithium lactate as substrate[12]. The enzyme activity was expressed as μ moles of formazan formed/mg protein/hour. *In vitro* effect of venom on enzyme activities was studied by the addition of 100 μg venom to tissue homogenate. *In vivo* studies were made during a period of 48 hours after sublethal doses of venom administration (intramuscular). One-third LD$_{50}$ was taken as sublethal doses. LD$_{50}$ was determined separately[13]. Statistical analysis of the data was done according to the method of Pillai and Sinha[14].

RESULTS AND DISCUSSION

Phosphorylase (*a* and *ab*) activity increased due to *H. fulvipes* venom both *in vitro* and *in vivo*. *In vitro* addition of 100 μg venom produced a rise in phosphorylase '*ab*' level to a greater extent than phosphorylase '*a*' in brain, liver and gastrocnemius muscle of frog (Table I). *In vivo* administration of sublethal doses of *H. fulvipes* venom produced a steep rise in phosphorylase (*a* and *ab*) activity in liver even by 6 hours after venom administration while the increase was not significant in brain and muscle at that time (Fig. 1). There was a lag in the increase of activity upto 6 hours in brain and then there was a sudden

TABLE I

In vitro *effect of* H. fulvipes *venom* (100 μg) *on phosphorylase* (a *and* ab) *and LDH activity levels in brain, muscle and liver of frog*

		Phosphorylase (μ moles of Pi/mg protein/hr) a	Phosphorylase (μ moles of Pi/mg protein/hr) ab	LDH activity (μ moles of formazan/mg protein/hour)
Brain	Normal	10·6±1·3	19·6±2·6	0·476±0·039
	Expt.	11·9±1·5	25·4±1·9	0·689±0·074
	% Change	12·3	29·6	44·65
Muscle	Normal	48·2±4·4	96·9±8·5	0·32±0·031
	Expt.	62·0±5·8	130·8±9·6	0·45±0·052
	% Change	28	35	41·5
Liver	Normal	18·9±3·9	38·8±6·3	0·619±0·098
	Expt.	24·6±4·2	53·54	0·886±0·123
	% Change	29·9	38	43·09

N.B.: Values represented are mean ± S.D. of eight separate experiments.

TABLE II

Per cent change of glycogen and lactic acid content in brain, muscle and liver of frog during 48 hours period after H. fulvipes *venom poisoning*

Time period	Glycogen			Lactic acid		
	Brain	Muscle	Liver	Brain	Muscle	Liver
6 hrs	− 1·8*	−32·2	−19·9	−4·3*	−54·3	+ 0·7*
12 hrs	−11·7*	−43·15	−36·0	−6·9*	−66·1	+ 2·2*
24 hrs	−17·7	−64·25	−51·9	−20·5	−85·9	+13·6
36 hrs	−14·8	−71·9	−44·0	−26·4	−84·48	+22·9
48 hrs	− 4·6*	−67·9	−41·0	−22·4	−87·3	+10·5

N.B.: + indicates increase.
− indicates decrease.
Eight individual experiments were done for each value and percentage was calculated from the corresponding mean.
* Not significant at 5% level ($P < 0·05$).

increase of activity level of phosphorylase *ab*. The increase in enzyme activity reached maximum by 24 hours and then the activity dropped towards normal even though remained significantly at an elevated level above normal in liver and muscle. Glycogen content decreased gradually upto 24 hours in brain and liver (Table II). In muscle maximum decrement (71·9%) occurred at 36 hours after venom poisoning. In brain there was an insignificant decrease (1·8%) at 6 hours and in liver and muscle it was significant ($P < 0·001$) even at 6 hours after venom poisoning. In necrotic muscle and liver glycogen content remained at a significantly decreased level even after 48 hours, though there was a tendency of returning to normal level.

The increase in phosphorylase activity was specific (true increase in enzyme level) since the soluble protein content of liver and brain was unchanged and increased in necrotic gastrocnemius muscle after venom poisoning[1]. In spite of the greater decrease of glycogen content of muscle, increase of phosphorylase (*a* and *ab*) was less in muscle compared to liver. This lower increase of enzyme activity in muscle may be apparent because the protein content increased in muscle which may be the cause of the low increase in muscle phosphorylase. The decrease in glycogen content and increase in phosphorylase were more in liver and muscle than in brain. These results suggest epinephrine-like action of venom. It was known that epinephrine stimulates cyclic AMP production which in turn stimulates the conversion of inactive phosphorylase *b*-kinase to active phosphorylase and promotes glycogen breakdown[15]. Circulating epinephrine induce hepatic and muscle glycogenolysis. Epinephrine and glucogen-like action of *L. quinquestriatus* venom was suggested previously[6]. It was also known that calcium was more a terminal signal for hormonal action than cyclic AMP[19]. *H. fulvipes* venom administration resulted in gradual increase of calcium in tissues[1] which may also promote glycogenolysis by increasing phosphorylase activity.

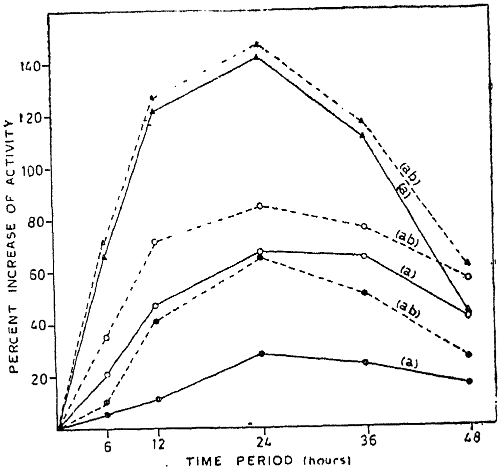

FIG. 1. Per cent increase of phosphorylase (*a* and *ab*) activity after *H. fulvipes* venom poisoning during 48 hours period in brain, muscle and liver of frog.

●——● = Brain
○——○ = Muscle
▲——▲ = Liver
ab = Total phosphorylase
a = Active phosphorylase

Lactic acid decreased in muscle and brain and increased in liver (Table II). Lactate dehydrogenase activity (Fig. 2) increased significantly ($P < 0{\cdot}001$) even by 6 hours reaching maximum by 12 hours (24 hours in liver) and then there was a gradual tendency towards normalcy. However it remained at an increased level even 48 hours after venom administration (Fig. 2). LDH activity was known to increase during conditions favouring anaerobic respiration to meet the energy demands when aerobic oxidations are lowered[15]. It was also known that scorpion venom causes myocardial damage[16] which is likely to rise LDH activity level both in tissues and in serum. *H. fulvipes* venom suppresses Kreb's cycle enzyme activity levels both *in vivo* and *in vitro*[1] conditions and decreases oxygen consumption[17]. It was also reported that *T. serrulatus* venom produced respiratory arrythmias[18].

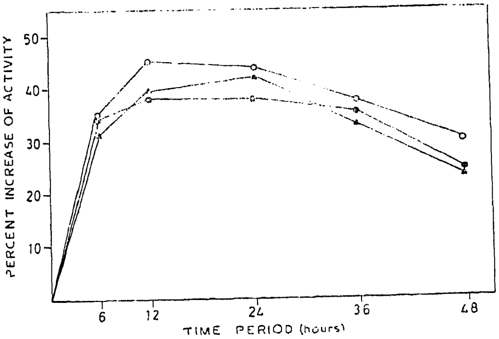

FIG. 2. Per cent increase of lactate dehydrogenase activity after *H. fulvipes* venom poisoning during 48 hours period in brain, muscle and liver of frog.

●——● = Brain
○——○ = Muscle
▲——▲ = Liver

The increase in LDH activity which utilizes NAD also alters NAD/NADH ratio which affects the energy metabolism. Since almost all oxidoreductions share a common pool of NAD/NADH, any alteration in the level of this ratio will affect the overall oxidoreduction processes of the cell[20]. Hence it is likely that venom administration may induce anaerobiosis in the cellular environment thereby altering normal energy metabolism.

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1. Venkateswarlu, D., *Ph.D. Thesis,* submitted to S.V. University, Tirupati, India, 1976.
2. Prameelamma, Y., Reddy, A. V. G. and Swami, K. S., *Toxicon,* 1975, 13, 482.
3. Rabia, F., El-Asmar, M. F. and Ibrahim, S. A., *Ibid.,* 1972, 10, 87.
4. Moustafa, F. A., Ahmed, N. Y. and El-Asmar, M. F., *Ibid.,* 1974, 12, 237.
5. Nair, R., Bhaskaran, and Kurup, P. A., *Indian J. Biochem. Biophys.,* 1973, 10, 133.
6. Mohamed, A. H., Hani-Ayobe, M., Besharoun, M. A. and El-Damarawy, N. A., *Taxicon,* 1972, 10, 139.
7. Watt, D. D., Donald, R. Babin and Ronald, V. Mlejnek, *Agricultural and Food Chemistry,* 1974, 22, 43.

8. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, 193, 265.
9. Carrol, N. V., Longley, R. W. and Roe, J. H., *Ibid.*, 1956, 22, 583.
10. Barker. and Summerson, as given in *Hawk's Physiological Chemistry*, by Oser, 14th edition, p. 1103.
11. Cori, G. T., Illingworth, B. and Keller, P. G., In: *Methods in Enzymology*, Vol. 1, Eds. S. A. Colowick and N. O. Kaplan, Academic Press, New York, p. 220.
12. Srikantan, T. N. and Krishna Murthy, C. R., *J. Sci. Industr. Res.*, 1955, 14, 20.
13. Reed, L. J. and Muenchi, H., *Amer. J. Hyg.*, 1938, 27, 493.
14. Pillai, S. K. and Sinha, H. C., In: *Statist cal Methods for Biological Workers* (Ra prasad and Sons, Agra, 3), 1968.
15. Harold, A. Harper, In: *Review of Physiolog cal Chemistry*, 14th edition, Lange Medic Publications, Maruzen Company Ltd., 196
16. Yarom, R. and Braun, K., *Toxicon*, 197 8, 41.
17. Venkatachari, S. A. T., Das, P. M. K. an Babu, K. S., *Ibid.*, 1970, 9, 199.
18. Freire-Maia, L., Ribeiro, R. M. and Berald W. T., *Ibid.*, 1970, 8, 307.
19. Latner, A. L., Cantarow and Trump *Clinical Biochemistry*, 7th edition, W. Saunders and Co., Philadelphia, 1975.
20. Atkinson, D. E., *Enzymes* (Ed. Boyer, P. D. Academic Press, New York, 1970.

EFFECT OF THALLIUM INTOXICATION ON THE AMINOACID CONCENTRATION OF DIFFERENT REGIONS OF THE RAT BRAIN

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ABSTRACT

Rats were given thallous acetate (5 mg/kg) i.p. for one week. The brain was removed and the free aminoacid levels were estimated in three regions; cerebral hemispheres, cerebellum and brainstem. Whereas the concentrations of taurine, serine, glycine, glutamic acid, histidine, tryptophan and phenylalanine was significantly reduced in the cerebral hemisphere, the levels of taurine, aspartic acid, lysine, phenylalanine, glutamic acid, tryptophan, histidine and GABA were diminished in the brainstem. All the aminoacids tested exhibited significant reduction in the cerebellum except histidine. Taurine, glutamic acid and tryptophan levels were uniformly diminished but other aminoacids showed regional heterogeneity.

INTRODUCTION

THE toxicity of thallium was recognized in man and animals[1] shortly after its discovery by Crookes in 1861[2]. The cases of human thallium poisoning were first reported in the 1920s from Germany but others were subsequently reported from Holland and Belgium[3]. Reports of thallitoxicosis with varying presentation still appear sporadically[3-6]. The initial symptoms of thallium intoxication are frequently neurologic and include dysesthesia, neuropathy, choreoathetosis, psychoses, excitement, convulsions, hysterical laughter, nerve palsies, coma and death[4,7]. The patho-physiology of thallium intoxication is, as yet, not clearly understood. Significant diminution of aminoacid concentration of brain areas has recently been reported by several workers in cases of convulsive disorders, including drug-induced convulsions[8]. It would, therefore, be appropriate to investigate the effect of thallium on the free aminoacid content of different regions of the rat brain. The present study is the first attempt in this direction. In this paper, the effect of thallium intoxication on the concentrati of taurine, GABA, glycine, phenylalanine, lysin serine, aspartic acid, glutamic acid, histidine an tryptophan in the cerebral hemisphere, brainster and cerebellum will be presented.

EXPERIMENTAL PROCEDURE

Male albino rats weighing 150–200 g, wer used. They were kept under standard laborato conditions throughout the experiment. Thallo acetate and other chemicals of Analar grade wer obtained from B.D.H. (England). The treated an control groups consisted of equal number o animals. Rats of the treated group were injecte thallous acetate 5 mg/kg, i.p. (0·1 ml/100 g bod weight of a 5 mg/ml solution) daily for 7 day The control group was treated in a similar manne with 0·1 ml/100 g body weight of physiologica saline. The rats were sacrificed by decapitation the brains were removed within 15–20 seconds an blotted on filter paper. Cerebral hemisphere brainstem and the cerebellum were dissected.

Assay of Aminoacids

The three different parts of the brain were weighed separately and homogenized in 80% ethyl alcohol. The homogenates were treated according to the method of Awapara[9] for the estimation of free aminoacids. Following storage for an hour on ice, the homogenates were centrifuged at 16,000 g on the International Refrigerated Centrifuge for 10 min. The precipitate was washed with 3–5 ml ethyl alcohol and the supernatant was evaporated to dryness on the water bath at 70°–90° C. To the residue was added 1 ml distilled water, 2 ml methyl alcohol and 2 ml chloroform and the resultant solution centrifuged for 20 min. The supernatant was obtained which was applied to chromatographic paper as recommended by Awapara[9]. The value of amino acids was calculated in μ mol/g, wet weight of brain tissue. The data were analysed statistically using student '*t*' test and significant differences between the means of the treated and the control groups and the '*p*' values calculated.

Results and Discussion

Following the administration of thallous acetate to rats, signs such as irritability, ataxia and at times convulsions were observed. After 5 days the rats usually became lethargic and the motor activity was considerably diminished.

Table I shows the effect of thallous acetate on en aminoacids in the cerebral hemisphere, cerebellum and brainstem. In the cerebral hemisphere, the concentration of taurine and histidine were most significantly reduced ($P < 0{\cdot}001$), followed by the serine level ($P < 0{\cdot}01$), glycine ($P < 0{\cdot}05$), glutamic acid ($P < 0{\cdot}01$), tryptophan ($P < 0{\cdot}05$) and phenylalanine ($P < 0{\cdot}05$). The reduction of GABA, lysine and aspartic acid levels was insignificant. All the aminoacids were significantly reduced in the cerebellum except histidine. This uniformity in the reduction of aminoacid concentration was, however, not observed in the brainstem. Whereas the levels of taurine, lysine, aspartic acid, phenylalanine, glutamic acid, histidine, tryptophan and GABA were significantly diminished in this region, the reduction in the concentration of serine and glycine was insignificant. The present investigation was carried out in three different areas of the brain to reveal the regional characteristics of thallium toxicity. Regional heterogeneity was, however, apparent in the distribution as well as in the thallium-induced reduction in the concentration of GABA, aspartic acid, lysine and histidine. Free aminoacids are of considerable interest as the source from which proteins and neurohumours are synthesized and to which end-products of their degradation return. They also participate in the regulation of metabolic homeostasis and are part of ionic environment[10]. Significant changes in the concentration of aminoacids, according to Modak *et al.*[11], must reflect changes in the underlying process of their synthesis,

TABLE I

Concentration of aminoacid (μmol/gm wet weight of brain tissue) in different regions of rat brain (mean ± S.E.)

Aminoacid	Cerebral hemisphere		Cerebellum		Brainstem	
	Control (N=8)	Experimental (N=8)	Control (N=8)	Experimental (N=8)	Control (N=8)	Experimental (N=8)
GABA	9·73±1·26	6·25±0·97	26·26±2·93	10·20±2·31*	14·64±1·55	4·77±0·87†
Aspartic acid	5·74±0·75	3·99±0·60	20·81±2·03	9·35±1·65**	15·12±2·33	4·53±0·97**
Taurine	3·74±0·32	1·54±0·24†	17·41±0·22	5·08±0·48†	11·22±1·44	3·03±0·16†
Serine	4·61±0·47	2·37±0·28**	13·70±1·52	5·66±1·04**	11·64±0·95	4·13±0·47†
Lysine	2·69±0·34	1·75±0·41	12·36±1·98	3·55±0·13**	9·20±0·95	3·08±0·54**
Ph. Alanine	1·95±0·15	0·92±0·11*	6·16±0·69	2·81±0·11**	5·97±0·52	1·88±0·11†
Glutamic acid	1·52±0·08	0·92±0·08**	3·91±0·33	2·03±0·25**	4·09±0·29	2·01±0·28**
Histidine	1·18±0·03	0·77±0·06†	3·43±0·41	2·15±0·41	4·11±0·23	2·41±0·25**
Tryptophan	0·98±0·06	0·65±0·06*	2·49±0·21	1·33±0·17*	2·22±0·12	1·50±0·11*
Glycine	17·35±1·13	10·03±1·26*	65·71±9·06	29·91±2·40**	31·75±3·06	11·51±1·33†

* Indicates $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; † $P < 0{\cdot}001$.

uptake or degradation in different regions of the brain. Another possible reason for the decrease in the levels of aminoacids mentioned, could be their likely utilization for the production of energy in thallium-toxicosis. In the present study the level of taurine, but not those of GABA and aspartic acid, showed a significant reduction in the cerebral hemisphere, suggesting that the reduction in GABA and aspartic acid levels is small and highly discrete and the reduction in taurine level is large and widespread. The latter suggestion is further supported by the observation that the taurine levels have been significantly reduced in all the brain areas included in this study. Like taurine, also GABA and glycine are inhibitory transmitters[12]. On the principle that the brain very often functions through "inhibition of inhibition" or "disinhibition" a decrease in the level of inhibitory transmitters is likely to be associated with hyper-excitability of neuronal structures. An important pathway of tryptophan metabolism in animals is conversion to serotonin. Similarly, phenylalanine and tyrosine are precursors of epinephrine and norepinephrine in the rat[13]. Thus the known precursors of serotonin, norepinephrine and epinephrine are depleted in the case of thallium poisoning. It is apparent that thallium intoxication leads to a decrement in the concentration of aminoacids but this diminution is not uniform throughout the brain.

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1. Grandeau, L., *J. Anat. Physiol. Norm*
1864, 1, 378.
2. Crookes, W., *Chem. News*, 1861, 3,
3. Irivine, A. and Johnson, H., *Medico*
1974, 42, 76.
4. Bank, W. J., Pleasure, D. E., Suzu
Nigro, M. and Katz, R., *Arch.*
1972, 26, 456.
5. Cavanagh, J. B., Fullor, N. H.,
H. R. M. and Rudge, P., *Quart. J*
1974, 43 (170), 293.
6. Reinhardt, G., *Internat. Microform. J.*
Med., 1973, 8 (3), 51.
7. Patty, F. A., *Industrial Hygiene and Tox.*
Interscience Publishers, Inc., New
London, 1949, p. 675.
8. Kar, P. P. and Mateen, M. A., *Euro*
Pharmacol., 1974, 25, 36.
9. Awapara, J., *Arch. Biochem.*, 1948, 19.
10. Friede, R. L., *Topographic Brain Ch*
Academic Press, New York, London,
p. 366.
11. Modak, A. T., Stavinoba, W. B
Weintraub, S. T., *Arch. Intern. Pharm*
1975, 217, 293.
12. Curtis, D. R., *Proc. Intern. Union*
Sci., 1974, 10, 176.
13. Meister, A., *Biochemistry of the Amin*
Academic Press, New York, London,
2, 909.

AWARD OF RESEARCH DEGREES

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degree in Chemistry to Sri Y. Seshasayi; Ph.D. degree in Mechanical Engineering to Sri K. Lakshminarayana Chetty; Ph.D. degree in Zoology to Sri D. Venkateswarlu; Ph.D. degree in Geology to Sri K. S. Sudhakar.

Karnatak University, Dharwar, has awarded the Ph.D. degree in Chemistry to Shri Shivasharan Siddappa Kaddargi; Ph.D. degree in Mathematics to Smt. Pushpavati; Ph.D. degree in Zoology to Shri Gangadhar Basalingappa Dodakundi; Ph.D. degree in Mathematics to Shri Shivashankar Sidramappa Chetty; Ph.D. degree in Botany to Shri Ulhas Damodar Bongale.

Utkal University, Bhubaneswar, has awarde
Ph.D. degree in Physics to Shri Narayan
Das; Ph.D. degree in Mathematics to Shri Sure
nath Bastia; Ph.D. degree in Botany to Shri
Sinha.

Sambalpur University, Burla, Orissa, has
the Ph.D. degree in Chemistry to Sri
Chandra Roy.

The M.S. University of Baroda, has awarde
Ph.D. degree in Botany to Shri Jitendrak
Jayantilal Dave; Ph.D. degree in Biochemi
Shri M. N. Subba Rao; Ph.D. degree in Bota
Shri Sanjappa, Munivenkatappa; Ph.D. degre
Botany to Shri Rajendrakumar.

LETTERS TO THE EDITOR

THE CRYSTAL STRUCTURE OF MANGANESE MALONATE DIHYDRATE $C_3H_2O_4\cdot Mn, 2H_2O$

THE title compound was investigated to unravel the scheme of hydrogen bonds as well as to study the oxygen ligands around the manganese.

Synthesis.—The crystals of manganese malonate dihydrate were prepared by taking malonic acid and manganese carbonate in the ratio of 2 : 1; water being used as solvent. The concentrated solution was left for slow evaporation till needle-shaped crystals began to appear. The mother liquor was removed by decantation and the crystals were washed with alcohol and acetone.

Crystal data.—Orthorhombic with $a = 9\cdot55$, $b = 7\cdot40$, $c = 8\cdot37$ Å. $D_{calc} = 2\cdot17$, $D_{obs.} = 2\cdot14$ gm/cc, $Z = 4$. Space group absences could lead to $Pca2_1$ or Pcam. As there are only four formula units in the unit cell with the malonate residue unlikely to possess a mirror symmetry, space group $Pca2_1$ was assumed which has been confirmed by structure determination. Reflexions (460) were collected photographically using Weissenberg photography and intensities estimated visually. Usual corrections were applied and the data brought to absolute scale by statistical methods.

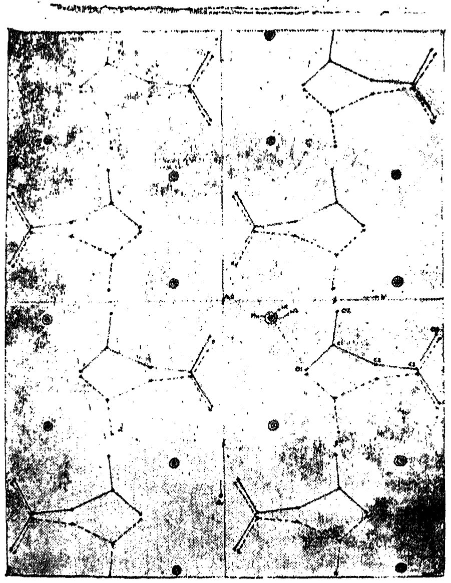

FIG. 1. Crystal structure looking down [001].

Crystal structure and comments.—The heavy atom was located from three-dimensional Patterson synthesis. The projection down the [001], being centrosymmetric, was solved by phasing the reflexions from heavy atom contribution. [001] Fourier was refined by series of successive syntheses. The *z*-coordinates were roughly located from packing considerations and adjusted by trial and error methods. At the present stage of analysis (with only overall isotropic temperature factor) R (hkO) $= 0\cdot16$, R (Okl) $= 0\cdot17$, R (hkl) $= 0\cdot18$.

A view of the structure down [001] is shown in Fig. 1. In the crystal, manganese is six-fold co-ordinated with Mn–O distances ranging from 2·08 Å to 2·22 Å. The water molecules and the surrounding oxygen atoms form a distorted octahedron. There are several short contacts between the oxygen atoms ranging from 2·82 Å to 3·20 Å, some of which may be short contacts between oxygen atoms of the polyhedra and some hydrogen bonds. As considerable interest lies in deciding the scheme of hydrogen bonds in the crystal, further least-squares refinement of the data is in progress.

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SALICYLIC ACID AND RELATED COMPOUNDS AS INDICATORS FOR THE DIRECT N-HYDROXY ETHYL ETHYLENE DIAMINE TRIACETIC ACID (HEDTA) TITRATION OF FERRIC IONS

No indicators have been reported for the direct titration of N-hydroxy ethyl ethylene diamine triacetic acid (HEDTA) with ferric ions. The present article reports the use of salicylic acid, sulphosalicylic acid and salicylamide as indicators for the direct HEDTA titration of ferric ions. All the three substances give violet colours with ferric ions. When such a solution was titrated with HEDTA the characteristic violet colour gave place to yellow at the end point.

Materials

Aqueous ferric nitrate solution (0·1 M) and HEDTA solution (2·78 g/l) were employed in this work. Indicator solution had 1 g indicator in 100 cc alcohol. The solution was stable for four weeks. Metal solutions had a concentration of 0·2 M.

Suggested Procedure

The ferric nitrate solution (5 ml of 0·01 M) is brought to pH 1·0 by adding dil. nitric acid and this is mixed with four drops of a 1% solution of the indicator and suitably diluted and titrated against standard HEDTA solution to a deep yellow colour. The results are compared with EDTA titrations. Good values are obtained in the pH range of 1–3.

Effect of Ferric Ion Concentration

Accurate results (error < ·5%) were obtained with 1·0 ml of the ferric nitrate solution in the concentration range from 0·01 M to 0·001 M. Thus micro titration of ferric ions with HEDTA is feasible with these indicators.

Effect of Foreign Ions

Synthetic solutions containing 2·8 mg iron (III) were titrated at pH 1·0. It was found that 100 fold excess of Na^+, K^+, NH_4^+, La^{3+}, Cs^+, Be^{2+}, UO_2^{2+}, Cl^-, Br^-, NO_3^- and SO_4^{2-}, 4 fold excess of Ca^{2+}, Ba^{2+} and Sr^{2+}, 2-fold excess of Zn^{2+}, Mg^{2+}, Al^{3+}, Mn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+} an equal amount of Pb^{2+}, Ag^+, Cr^{3+} and HCO_3^- could be tolerated (*i.e.*, error < 1%). The tolerance was increased to five times by masking in the case of Cu^{2+} by thiosulphate, in the case of Mn^{2+} by hydrogen peroxide and in the case of Zn^{2+} by glycerine. Phosphate, fluoride, oxalate, vanadate, molybdate and tungstate interfered at all levels.

Applications

The above procedure was applied to the determination of ferric ions in the analysis of pharmaceuticals containing iron in the form of colloidal iron hydroxide ferrous gluconate, ferrous fumarate, and ferrous sulphate (Table I). The bauxite ore was analysed by the suggested procedure. The expected iron content was 2·86% Fe_2O_3 while that found by the above procedure was 2·80% Fe_2O_3.

TABLE I

Analysis of drugs containing iron by direct titration with HEDTA

Name of drug	Fe^{3+} expected (g)	Fe^{3+} found (g) Salicylic acid	Salicylamide	Sulphosalicylic acid
Polycytol tablet (ferrous sulphate)	0·195	0·194	0·194	0·194
Iberol tablet (do.)	0·525	0·524	0·522	0·522
Iberol liquid (do.)	0·131	0·130	0·130	0·130
Livogen capsule (ferrous fumarate)	0·150	0·149	0·149	0·147
Microfer tablet (ferrous gluconate)	0·195	0·194	0·194	0·194
Tonoferon drops (ferric hydroxide)	0·050	0·047	0·046	0·046

We thank the authorities of Gujarat University for the award of a research scholarship to one of us (JVP).

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THERMAL DECOMPOSITION OF ZINC AND CADMIUM HEXAOXOIODATES

Introduction

SEVERAL reports have been published on the thermal decomposition of the lanthanide and other group III hexaoxoiodates[1-4]. However, there appears to be no data available in the literature concerning the thermal decomposition of zinc and cadmium hexaoxoiodates (VII). The purpose of this work is to study the thermal decomposition of the above-mentioned periodates by means of thermogravimetric and differential thermal analysis.

Experimental

Zinc and cadmium hexaoxoiodates (VII) ($M_2HIO_6 \cdot 1{\cdot}25\ H_2O$, where M = Zn, Cd) were prepared by mixing in stoichiometric proportion, equimolar dilute solutions of the reagent grade periodic acid and the respective metal acetates, followed by filtration and drying in a vacuum desiccator over P_2O_5. The dried and powdered compounds were chemically analysed for metal, iodine and water contents. Metal ions were analysed complexometrically. Iodine was analysed gravimetrically as AgI and water was determined by Karl Fischer method. The analytical results are given in Table I.

Differential thermal analysis of both periodates was carried out using 'Stanton Thermoanalyser' with a programmed heating rate of 10° C/min., in an air atmosphere with sample weights of 175 to 200 mg. The sample holder was a cylindrical platinum crucible and α-alumina served as a reference material. Thermogravimetric analysis of the compounds were carried out in air in a 'Stanton Mass Flow Thermobalance' with a programmed heating rate of 2° C/min. with sample weights ~ 500 mg.

Results and Discussions

Figure 1 shows the thermogravimetric and DTA curves of zinc and cadmium hexaoxoiodate hydrates ($M_2HIO_6 \cdot 1{\cdot}25\ H_2O$). It is evident from the TG curves that the first weight loss begins at 60° C and terminates at 210° C for zinc and at 275° C for cadmium hexaoxoiodate hydrate respectively. This weight loss

TABLE I

Analytical results of the thermal decomposition products

Products obtained	$Zn_2HIO_6 \cdot 1 \cdot 25\ H_2O$			$Cd_2HIO_6 \cdot 1 \cdot 25\ H_2O$		
	% Zn	% I_2	% H_2O	% Cd	% I_2	% H_2O
Starting product	34·56 (34·67)[a]	34·00 (33·65)	6·00 (5·97)	47·50 (47·69)	27·25 (26·94)	4·80 (4·79)
M_2HIO_6	36·80 (36·85)	36·00 (35·80)	..	49·95 (50·07)	28·45 (28·30)	..
$M_4I_2O_{11}$	37·80 (37·81)	36·90 (36·73)	..	50·90 (51·11)	29·00 (28·87)	..
MO	80·30 (80·34)	..	..	87·46 (87·52)	..	..

'*a*'—values in the parantheses are theoretically calculated values.

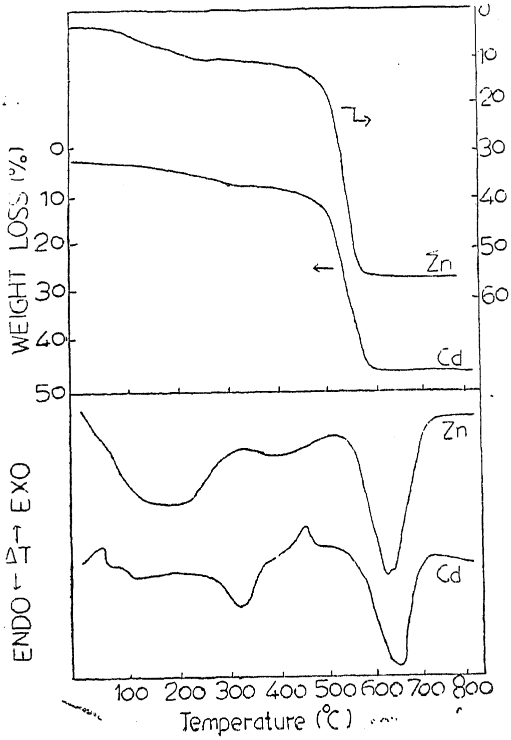

FIG. 1. TGA and DTA diagrams of $Zn_2HIO_6 \cdot 1 \cdot 25\ H_2O$ and $Cd_2HIO_6 \cdot 1 \cdot 25\ H_2O$.

corresponds to the release of one and a quarter molecules of water, in each case, and is in excellent agreement with the theoretically expected weight loss for the compounds. The associated flat endothermic peak for zinc is spaced in the temperature range 60° C to 210° C in the DTA diagram. The respective peak for cadmium lies in the range 60° C to 275° C and is hollow and broad. Between 210° C and 500° C the anhydrous hexaoxoiodates lose weight corresponding to half a water molecule per molecule, which is most probably the constitutional water leading to the formation of $M_4I_2O_{11}$[5]. Though this weight loss is not clearly indicated on the TG curve (being very small), the associated endothermic peaks, broad in the case of zinc and small but distinctly sharp at 315° C in the case of cadmium on DTA confirm the loss of constitutional water. The DTA curve of cadmium hexaoxoiodate, unlike zinc salt, exhibits a small, sharp and almost symmetrical exothermic peak at 445° C, without any accompanying weight loss. This is probably due to a change in the crystallinity of the compound. The final decompositions begin rapidly at 500° C and result in the sublimation of iodine and formation of respective metallic oxides. This weight loss is also associated with a big symmetrical endothermic peak at 600 to 625° C in both cases.

Based on the above results, the following mechanism can be proposed for the decomposition of these hexaoxoiodates, M being Zn or Cd.

(i) $M_2HIO_6 \cdot 1 \cdot 25\ H_2O \rightarrow M_2HIO_6 + 1 \cdot 25\ H_2O$
(ii) $2M_2HIO_6 \rightarrow M_4I_2O_{11} + H_2O$
(iii) $M_4I_2O_{11} \rightarrow 4MO + I_2 + 3\frac{1}{2}\ O_2$.

The confirmation of the above mechanism was achieved by complete chemical analysis of the intermediate products obtained by isothermal experiments at selected temperatures. The results are given in Table I.

Näsänen *et al.*[6], in their studies on the compound Cu (OH) $(H_2IO_6) \cdot H_2O$ for which Siebert[7] has later proposed the formula $Cu_2HIO_6 \cdot H_2O$, arrived at a similar conclusion that the compound decomposes to give $Cu_4I_2O_{11}$ as an intermediate stage.

The authors wish to thank the Head of the Chemistry Division of the Bhabha Atomic Research Centre, Bombay, for permitting the use of 'Stanton thermo-analyser' to them.

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1. Petru, F., Hradilova, J. and Hajek, B., *J. Inorg. Nucl. Chem.*, 1970, **32**, 1485.
2. Kyrki, J. R. and Lokio, A. H. J., *Suomen Kemistilehti*, 1971, **B44**, 105.
3. Lokio, A. H. J. and Kyrki, J. R., *Ibid.*, 1971, **B44**, 334.
4. —, *Finn. Chem. Lett.*, 1974, **1**, 5 and the references mentioned therein.
5. Partington, J. R. and Bahlu, R. K., *J. Chem. Soc.* (*London*), 1934, **1091**, 1771.
6. Nāsānan, R., Uggla, R. and Hrvonen, Y., *Suomen Kemistilehti*, 1957, **B30**, 31.
7. Siebert, H., *Fortschr. Chem. Forsch*, 1967, **8**, 470.

SPECTROPHOTOMETRIC STUDY OF THE COMPLEX BETWEEN IRON(111) AND β-RESORCYLIC ACID

THE use of β-Resorcylic acid (BRA) in the determination of metal ions has led to an increased interest in the chemistry of transition metal chelates. A survey of literature shows the chelation of this ligand[1–5] with UO_2^{2+}, Ni^{2+}, Al^{3+}, La^{3+}, Co^{3+} and V^{5+}. Although some potentiometric investigations[6] have been made on iron(111) and BRA complexes, detailed spectrophotometric studies are lacking. Since the reaction can be utilised for the estimation of iron in steel, a detailed investigation of the complexation between iron(111) and BRA based on spectrophotometric measurements has been undertaken.

TABLE I

Stability constants and free energy of formation of the complexes

λ_{max} nm	Composition	log Stability constants				ΔF° KJ mole^{-1}
		Job's method	Mole ratio method	Slope ratio method	Average	
530	1:1	7·114	7·176	..	7·145	40·77
502	1:2	..	11·286	11·295	11·290	64·37
482	1:2	..	11·367	11·389	11·378	64·92

Experimental

A 0·01 M β-resorcylic acid solution in 5% aqueous ethanol and 0·01 M ferric ammonium sulphate solution in 0·1 NH_2SO_4 were used. The pH measurements and spectrophotometric measurements were carried out with a Beckman pH meter and a Unicam SP 500 spectrophotometer respectively.

Results and Discussion

Instantaneous colour formation was observed on mixing BRA and Fe(111) solutions in the pH range 2–5 and the colour was found to be stable for more than a week. Spectral characteristics of the complex mixtures containing various proportions of iron(111) and BRA, the latter being always in excess, were studied. Maximum absorbance occurred at the 530 nm (pH 2–3), 502 nm (pH 3–4) and 482 nm (pH 4–5). Beer's law was found to be valid under these conditions.

Stoichiometry of the complex

Job's[7] method of continuous variation as modified by Vosburgh and Cooper[8], mole ratio method of Yoe and Jones[9] and the slope ratio method proposed by Harvey and Mannings[10] were employed. Stability constants of complexes and hence their free energy of formation ($\Delta F^{\circ} = -2 \cdot 303\ RT \log K$) were calculated.

Job's method

At 530 nm (pH 2–3), maximum absorption was observed at a Fe(111): BRA ratio of 1:1, while a 1:2 complex was noticed at 502 (pH 3–4) and 482 nm (pH 4–5). The formation of the complexes was also confirmed by mole ratio and slope ratio methods.

Stability constants of the complexes and the free energy changes of complex formation have been shown in Table I. The coordination of metal ion is assumed to take place through the ionized hydroxyl group (γ—to the carboxyl group) in the ligand[6].

Iron content of two standard samples of stainless steel (Mn-Cr steel, Mn 0·72% and Cr 1·18%; Cr-Ni steel, Cr 1·2%, Ni 4·07%) were determined by the present procedure. The method is accurate to ± 0·5%. Nickel, chromium and manganese do not interfere under these conditions.

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1. Reddy, M. L. N. and Seshaiah, U. V., *Ind. J. Chem.*, 1964, **2**, 34.
2. Das Gupta, P. N., *J. Ind. Chem. Soc.*, 1929, **6**, 763.
3. Dubey, S. S. and Dhindsa, S. S., *J. Inorg. Nucl. Chem.*, 1970, **32**, 543.
4. Dhindsa, S. S., Sharma, R. C. and Bhargava, D. N., *J. Ind. Chem. Soc.*, 1970, **53**, 526.
5. Desai, M. N. and Desai, M. N., *Ibid.*, 1965, **42**, 643.
6. Jatkar, S. K. K. and Mattoo, B. N., *Ibid.*, 1953, **30**, 775.
7. Job, P., *Ann. Chim.*, 1928, **10**, 113.
8. Vosburgh, W. C. and Cooper, G. R., *J. Amer. Chem. Soc.*, 1941, **63**, 437.
9. Yoe and Jones, *Ind. Eng. Chem.* (*Anal. Ed.*), 1944, **16**, 111.
10. Harvey, A. E. and Mannings, D. L., *Ibid.*, 1930, 72, 4488.

FORMATION CONSTANTS OF SOME LANTHANIDE-CHELATES OF *o*-(N, 2-HYDROXY-α-METHYLBENZYLIDENE IMINO) BENZOIC ACID

A SURVEY[1-3] of the literature has indicated that no work has been done on La(III), Ce(III), Pr(III), Nd(III), Sm(III) and Gd(III) chelates of *o*-(N, 2-hydroxy-α-methylbenzylidene imino) benzoic acid (H_2BB) Schiff base derived from *o*-hydroxyacetophenone and *o*-aminobenzoic acid. Hence the work on the potentiometric studies of these chelates was undertaken. The measurements were carried out by Irving-Rossotti method[4] at 25°, 30° and 35° C in aqueous media ($\mu = 0 \cdot 1, 0 \cdot 05$ and $0 \cdot 01$ M $NaClO_4$).

Precision pH-meter type OP : 205 No. 837 with glass-calomel assembly was used. H_2BB was synthesised by the general procedure reported earlier[5], m.p. 182° C, found : C, 70·52 ; H, 4·96 ; N, 5·38 ; calcd. for $C_{15}H_{13}NO_3$, C, 70·58 ; H, 5·09 ; N, 5·49%. All the chemicals used were either BDH or Ridiel reagents. The standard solutions of H_2BB, metal-nitrates and sodium perchlorate were prepared in doubly-distilled water.

The ligand solution (0·01 M) was prepared in water. For the evaluation of thermodynamic constants, experiments were carried out in media of low ionic strengths (0·1 M, 0·05 M, 0·01 M) from these, an extrapolation to zero ionic strength was possible. The following solutions (total volume 25·0 ml) were titrated against the standard carbonate-free sodium hydroxide (0·1 M) and the titration curves had the usual shapes. (1) 5·0 ml, 0·01 M $HClO_4$ + 2·5 ml, 1·0 M $NaClO_4$ + 17·5 ml H_2O ; (2) 5·0 ml, 0·01 M $HClO_4$ + 2·5 ml 1·0 M $NaClO_4$ + 10·0 ml 0·01 M H_2BB + 7·5 ml H_2O and (3) 5·0 ml, 0·01 M $HClO_4$ + 2·5 ml 1·0 M $NaClO_4$ + 10·0 ml 0·01 M H_2BB + 2·0 ml metal-ion-solution + 5·5 ml H_2O.

The results of the dissociation constants of H_2BB and the stability constants of its lanthanide-chelates were obtained at different ionic strengths at 25°, 30° and 35° C. The formation curves of the metal-chelates suggest the formation of 1 : 2 complexes. The stabilities of the metal chelates follow the order, La(III) < Ce(III) < Pr(III) < Nd(III) < Sm(IIII) and < Gd(III), which is in accordance with lanthanide contraction.

The thermodynamic stabilities (Table I) have been obtained by extrapolation of measured formation constants to zero ionic strength from graph between log of stability *vs* μ, where μ is the ionic strength.

TABLE I

Thermodynamic stabilities and free energies of H_2BB and its lanthanide-chelates

Ligand metal chelates	at 25°		at 30°		at 35°	
	log K°	−ΔG° Kcal/mole	log K°	−ΔG° Kcal/mole	log K°	−ΔG° Kcal/mole
H_2BB	20·40	1·783	20·35	1·810	20–30	1·835
La(III)	12·10	1·463	12·00	1·480	11·90	1·509
Ce(III)	14·00	1·554	13·85	1·570	13·70	1·596
Pr(III)	14·80	1·576	14·65	1·590	14·50	1·625
Nd(III)	15·25	1·608	15·15	1·625	15·00	1·651
Sm(III)	15·80	1·629	15·65	1·645	15·50	1·670
Gd(III)	16·30	1·718	16·20	1·695	16·00	1·685

Harned[6-8] gave the relation between log K_1^H and temperature as $(pK^H - ct^2) = -2c\theta t + (pK_m^H + c\theta^2)$ where $pK^H = -\log K^H$ at $t°$ C; pK_m^H is minimum pK^H value at $\theta°$ C and c is a constant having the value $5 \cdot 0 \times 10^{-5}$ deg^{-2}. A plot of $(pK^H - ct^2)$ *vs* t must be a straight line having a slope of $-2c\theta$ and an intercept $(pK_m^H + c\theta^2)$ at $t = 0$, and this was found true in the present case. The values of θ and pK_m^H were calculated (Table II). ΔH values were also calculated using Harned's equation, $\Delta H = 2 \cdot 303 \times 10^{-4}\ RT^2\ (t - \theta)$.

TABLE II

Dissociation constants and the thermodynamic parameters at different temperature and pK_m^H and ΔH calculated from Harned equation for H_2BB at $\mu = 0 \cdot 1$ M

Temp. (° K)	pK^H	$-\Delta G$ Kcal/mole	θ° C	pK_m^H	$-\Delta H$ (from Harned's equation)
298	20·27	1·783	100	20·00	3·048
303	20·23	1·810			2·941
308	20·19	1·835			2·822

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1. Cotton, F. A., *Prog. Inorg. Chem.*, 1966, 7, 88.
2. Hodgson, D. J., *Ibid.*, 1975, 19, 1.
3. Syamal, A., *Coord. Chem. Rev.*, 1975, 16, 309.
4. Irving, H. and Rossotti, H. S., *J. Chem. Soc.*, 1945, p. 2904.
5. Mehta, R. K. and Gupta, R. K., *Ind. J. Chem.*, 1973, 11, 56.
6. Harned, H. S. and Ehlera, R. W., *J. Am. Chem. Soc.*, 1933, 55, 652.
7. — and Kazanjian, *Ibid.*, 1936, 58, 1912.
8. — and Embree, N. D., *Ibid.*, 1934, 56, 1050.

TITRIMETRIC DETERMINATION OF 2-MERCAPTOETHANOL, MERCAPTOACETIC ACID AND 2-MERCAPTOPROPIONIC ACID WITH POTASSIUM PERIODATE

MERCAPTANS or thiols, on oxidation yield disulphide links —S—S—, which hold together different polypeptide chains[1]. In the present investigation we have examined the behaviour of potassium periodate as an oxidimetric reagent for the estimation of 2-mercaptoethanol, mercaptoacetic acid and 2-mercaptopropionic acid. It was found that these mercaptans are oxidized stoichiometrically at room temperature with a single electron change at neutral pH, to their corresponding disulphides.

$$2\,RSH + IO_4^- \longrightarrow R\text{-}S\text{-}S\text{-}R + IO_3^- \rightleftharpoons H_2O$$

Back titration procedure has not been adopted as the reaction was fast enough to enable a direct titration with a visual or potentiometric end points.

Materials and Methods

Aqueous potassium periodate (0·01 M) was standardized iodimetrically and by arsenite titration[2]. Mercaptan samples were gifts from Evans Chemitics, New York. All other chemicals were of reagent grade.

Recommended Procedure

A sample containing 0·1 – 1·0 m mole of mercaptan was weighed in a 150 ml Erlenmeyer flask and dissolved in 50 ml of water after which 2 g of sodium hydrogen carbonate was added together with 100 mg of potassium iodide and 2 ml of 1% starch solution. The end point was determined by blue colour of starch iodide, formed by the excess drop of the periodate reagent.

Results and Discussion

Solutions of 2-mercaptoethanol, mercaptoacetic acid and 2-mercaptopropionic acid were standardized with Hg(II)[3], Cu(II)[4] and iodine[5] respectively with an average deviation of 0·6%, 0·3% and 0·1% respectively. Quantitative results obtained for the determination of 2·22–46·50 mg of 2-mercaptoethanol were accurate to 0·6% (av. dev. 0·5%), for 1·62–53·80 mg of mercaptoacetic acid and 1·71–51·48 mg of 2-mercaptopropionic acid the accuracy was found to be 0·5% (av. dev. 0·3%).

Compounds which do not interfere when present in small amounts include thiophene (10 moles of substances: 1 mole of —SH compound), urea (8 : 1), methyl acrylate (5 : 1), diacetone alcohol (10 : 1). Glucose and fructose do not interfere even when present in large amounts. Large interferences are noticed with thiocarbonyl compounds and sulphocyanide ions. The method is inapplicable to mercaptans having free β-carboxyl group due to the susceptibility to further oxidation[6]. From the titration of 2-mercaptoethanol it is evident that the compound is preferentially oxidized to disulphide rather than undergoing Malaprade reaction.

Potassium chlorate and potassium chlorite have also been used as oxidizing agents in acidic medium for the determination of mercaptans. Potassium perchlorate unlike periodate could not be used as an oxidizing agent because of its weak oxidizing power and low solubility in water.

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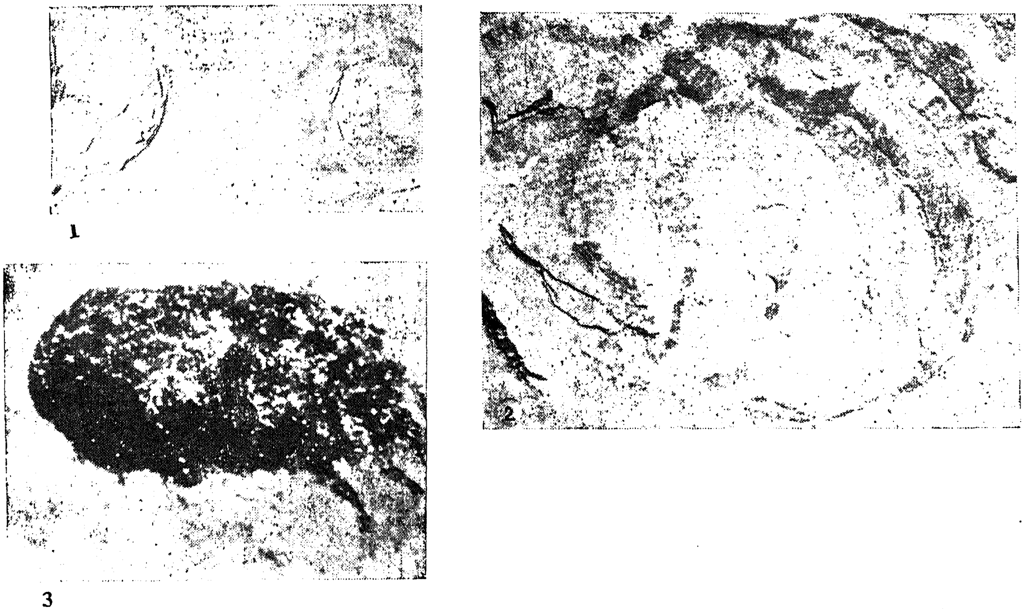
1. Ahmed, J. and Bose, S., *J. Indian Chem. Soc.*, 1973, **50**, 505.
2. Berka, A., Volterin, J. and Zyka, J., *Newer Redox Titrants*, Pergamon, Oxford, 1965, p. 67.
3. Fritz, J. S. and Palmer, T. A., *Anal. Chem.*, 1961, **33**, 98.
4. Sant, S. B. and Sant, B. R., *Ibid.*, 1959, **31**, 1879,
5. Kimball, J. W., Kramer, R. L. and Reid, E. E., *J. Am. Chem. Soc.*, 1921, **43**, 1199.
6. Danehy, J. W. and Oester, M. Y., *J. Org. Chem.*, 1967, **32**, 1491.

NEW FOSSIL FINDS FROM THE LOWER VINDHYAN ROCKS (PRECAMBRIAN) OF CENTRAL INDIA

THIS note records definite evidence of worm and arthropod remains from the Lower Vindhyan rocks of Katni area, Madhya Pradesh. More than 200 worm and a lone arthropod remains are collected from an abandoned limestone quarry about 2 km, south-west of Katni railway station from the Rohtas Limestone.



FIGS. 1–3. Fig. 1. Annelid remains, × 0·8 (approx.). Fig. 2. Annelid remains × 3·8 (approx.). Fig. 3. Arthropod (insect) remain, × 18·2 (approx.).

The worm remains have been classified under the phyllum Annelida and appear to be a new genus (Figs. 1 and 2). They are coiled, bent on themselves and segmented. The arthripod remain is carbonised, laterally preserved and classified under class Insecta, on the basis of the presence of head, thorax with three jointed legs and segmented abdomen (Fig. 3). It is the first record of insect from Precambrian of India and possibly from the world.

The discovery of arthropod and worm remains from the Rohtas Limestone will throw a new light on the evolution of arthropod and annelids which must have evolved from primitive forms in still older rocks.

Detailed work is in progress and systematics will be published shortly (Tandon and Kumar[1]).

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1. Tandon, K. K. and Kumar, S., *Geophytology*., 1977 (In press).

AIR-BREATHING IN THE CATFISH, *MYSTUS VITTATUS* (BLOCH)

THE catfish, *Mystus vittatus* (order : Physostomi; sub-order : Siluridae; family : Bagridae) inhabits tropical ponds and rivers and is not known to leave water. It has no well defined accessory air-breathing organs. However, if kept out of water, the fish survives for about 5 to 8 hours under laboratory conditions (28° C;

80% R.H.). This appears to indicate that the fish has some air-breathing ability.

The present study is undertaken to assess the air-breathing potentiality of the fish. Oxygen consumption in air was measured using a manometer (adopted from Umbreit *et al.*[6]). Oxygen obtained from air and water, when the fish was kept in water with free access to air, was determined using a respiratory chamber[5] which contains water with an accessible gas phase above. Oxygen consumption from water was determined by estimating the loss of oxygen using Winkler's method (Welsh and Smith[7]) and oxygen consumed from air was determined using a manometer connected to the gas phase. Oxygen consumption under water with no access to air was also estimated using the method followed by Job[3]. All measurements were made at 28 ± 1° C and the results are presented in Table I.

TABLE I

Oxygen consumption of Mystus vittatus (*ml/kg/hr ± S.E.*)
N = 12

	From water	From air	Total
Oxygen consumption			
from air	..	38·65± 3·48	38·65± 3·48
from water with access to air	60·91± 3·40 (67%)	29·43± 1·14 (33%)	90·34± 3·19
from water without access to air	116·81± 12·53	..	116·808± 12·53

From Table I, it is clear that *Mystus vittatus* utilizes atmospheric oxygen both when it is in air and in water with access to air. Under natural condtions, the fish living in water with free access to air appears to utilize atmospheric oxygen to supplement aquatic breathing. Higher metabolic rate of the fish kept under water with no access to air might be suggested to imply a stress factor under forced conditions of submersion.

Mystus vittatus is a physostomous fish, with air-bladder opening by a small duct on the dorsal surface of the oesophagus. Whether the air-bladder contributes to aerial gas exchange is not proved. The air-bladder serving a respiratory function is not unknown among teleosts.[4] *Mystus vittatus* in aquarium tanks swims slowly in an inclined position in contact with the surface film of water with its mouth occasionally projecting beyond the surface into the air. Air bubbles are sometimes expelled through the opercular openings. The fish appears to lock up a certain amount of air in the oral cavity to enrich the water with oxygen and/or to facilitate direct diffusion of oxygen through the surface of the oral cavity. Whether this is a means of utilizing atmospheric oxygen in times of need is not establshied. The possibility of the skin and gills serving in aerial gas exchange when the fish is kept out of water, as reported in other air-breathing fishes[1,2], may not be totally ruled out Further investigations on the routes of aerial gas exchange are now in progress.

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1. Carter, G. S. and Beadle, L. C., *J. Linn. Soc.*, 1931, **37**, 327.
2. Dubale, M. S. *Proceedings of the First Summer School of Zoology* (Simla, 1961), A review, 1963 p. 325.
3. Job, S. V., *Pubs. Ont. Fish. Res. Lab.*, 1955, **73**, 1.
4. Jones, F. R. H. and Marshall, N. E., *Biol. Rev.*, 1953, **28** (1), 16.
5. Reddy, T. Gopalakrishna and Natarajan, G. M., *J. Annamalai Univ. Sci.*, 1970, **28**, 155.
6. Umbreit, W. H., Burris, R. H. and Stauffer, J. F., Burgess Publishing Company, Minneapolis, 1959.
7. Welsh, J. H. and Smith, R. L., *Ibid.*, 1960.

NEW RECORD OF FRESHWATER OLIGOCHAETE *AULOPHORUS FLABELLIGER* STEPHENSON, 1930, FROM INDIA

DURING the course of our investigations on the aquatic oligochaete fauna of Guntur District, Andhra Pradesh, we came across a species of the family Naididae, *Aulophorus flabelliger*, Stephenson, which hitherto has not been reported from India. This species was previously described by Stephenson (1930)[2] from Lake Naivasha, Kenya. Of a total of 16 valid species known under the genus *Aulophorus*, only six were reported from India till now (Naidu, 1962)[1].

We have collected this species along with *Aulophorus tonkinensis* (Vejdovsky, 1894), a closely related tube dwelling form, from a semi-permanent freshwater pond at Moparru, situated 35 km south-east of Guntur, associated with the roots of *Pistia* sp. Hydrographic conditions prevalent at the time of collection are, pH 8·0, air temperature 36·6° C, water temperature 34·5° C; water turbid, alkalinity 237 ppm, dissolved oxygen 2·83 ppm. Only two worms were collected.

Worms small, delicate, transparent inhabiting tubes of plant material, 3·5 mm long, body diameter 0·32 mm, total number of segments (*s*) = 26 + undifferentiated region; number of segements behind which budding zone develops (n) = 16.

Dorsal setae from VI, 1 hair and 1 needle per bundle. Hairs simple, 90–98 μ long, less than the diameter of the body. (Hairs 110–115 μ in original description). Needles 52–60 μ long with distal end expanded web-like with a maximum breadth of 15 μ, its free margin with 9 indentations. Figure of the needle closely resembles that given by the original author. Ventral setae of II–V 5 per bundle, slighly. curved, 110–118 μ long, nodulus proximal (D : P : : 25 : 13–24 : 13), prongs equally thick, distal longer than the proximal. Ventral setae from VI onwards 4 per bundle, with shaft proximally more curved than those of II–V, 52–55μ long with distal nodulus (D : P : : 6 :11-5 : 12; proximal prong longer and 3 times thicker than the distal.

Posterior end of the worm bears a branchial organ, cup-shaped, with a pair of cylindrical palps and 3 pairs of digitiform gills, ventral pair being the longest.

Alimentary canal shows distinct stomachic enlargement in IX.

Single budding zone is observed in one worm, clitellum is absent.

One of us (KSB) thanks the C.S.I.R. for awarding a Junior Research Fellowship.

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1. Naidu, K. V., *J. Bombay Nat. Hist. Soc.*, 1962, 59 (3), 897.
2. Stephenson, J., *Proc. Zool. Soc., Lond.*, 1931, 1-2, 33.

ON NEMATODE CLEAVAGE PATTERN WITH SPECIAL REFERENCE TO *TANQUA ANOMALA* (V. LINSTOW, 1904) (GNATHOSTOMATIDAE) FROM THE WATER SNAKE *NATRIX PISCATOR* SCHNEIDER

IN the embryology of nematodes, there are certain unique and characteristic features in respect of early cleavage stages which are of general interest. Detailed work on the embryology of nematodes has been done by several investigators. Work on the embryology of nematodes has been reviewed by Nigon[1] and much information has been given by Bird[2]. Parasitology is becoming increasingly popular in the curriculum of Indian Universities and in the search for interesting and easily available teaching models, it is found that the nematode *Tanqua anomala* from the stomach of the water snake *Natrix piscator*, forms good material.

Thus, the embryology of *Tanqua anomala* is a suitable model to project the characteristic cleavage pattern, resulting in a three celled stage and the characteristic 'T'-shaped condition in the four celled stage. The egg is oval with a thin and transparent egg shell allowing easy observation of the early stages of the cleavage. The first cleavage was observed in 1–2 hours after egg laying, dividing the egg into a bigger S1 cell and smaller P1 cell (Fig. 1 A). The S1 cell divides next and a three celled condition occurs. After this P1 cell divides and the typical 'T'-shaped condition is reached (Fig. 1 B).

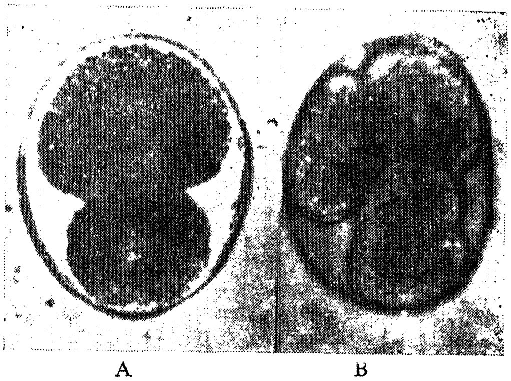

FIG. 1. Cleavage of the nematode *Tanqua anomala* egg. A, Two celled stage; B, Four celled stage; 'T'-shaped condition.

In general the first cleavage in nematodes results in the formation of two cells, a somatic cell S1 and a parental germ cell P1. In some cases, at this stage, the cells are unequal, the S1 cell being bigger than the P1 cell as in *Ditylenchus dipsaci*[3] or as in *Rodopholus similis*[4]. In the next stage S1 usually divides longitudinally whereas P1 divides transversely so that a characteristic 'T'-shaped condition is displayed. However, sooner or later a rearrangement of cells occurs and a rhomboid shape becomes evident. A slight delay in the division of P1 may result in a three celled condition for some time. In *Ditylenchus destructor*[5] or in *Meloidogyne naasi* one blastomere may not divide until the second one has divided twice resulting in a five celled stage. The interesting case is that of *Tylenchorhynchus claytoni* where Wang[7] reported that at the two celled stage S1 and P1 are almost equal in size. S1 divides into 'A' and 'B' cells. The 'A' cell divides obliquely into 'α' and 'a' cell. There occur three divisions of S1 cell at this stage but P1 divides much later. There are cases where although a three celled condition is reached, the cells lie in a linear fashion as in metastrongyloid *Dictyocaulus filaria*[8] or protostrogyloid *Varesstrongylus pneumonicus*[9]. In *T. anomala* at the three celled stage the blastomeres lie in a triangular condition.

One of us (BVK) thanks U.G.C. for the award of Junior Research Fellowship.

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---

1. Nigcn, V., *Traite de Zoologie*. Messon Press, 1965.
2. Bird, A. F., *The Structure of Nematodes*, Acad. Press, 1971.
3. Yuksel, H. S., *Nematologica*, 1960, **5**, 43.
4. Van Weerdt, L. G., *Ibid.*, 1960, **5**, 43.
5. Anderson, R. V. and Darling, H. M., *Proc. Helminth. Soc. Wash.*, 1964, **31**, 240.
6. Siddiqui, I. A. and Taylor, D. P., *Nematologica*, 1970, **16**, 133.
7. Wang, L. H., *J. Nematol.*, 1971, **3**, 101.
8. Dorani, V. P., *Ind. J. Helminnthol.*, 1965, **27**, 85.
9. —, *Ibid.*, 1965, **27**, 89.

INCIDENCE OF 'SPRING DWARF' DISEASE OF STRAWBERRY FROM INDIA

CONCERTED efforts are being made to popularise strawberry cultivation and also to establish th nurseries in plains of the Punjab State. Recently a large number of plants of different varieties, *viz.*, Gorella, Bangalore, Ped, Clomax, Black rose and Dilpasand brought from Chail, Simla Hills (H.P.), maintained in nurseries at Punjab Agricultural University, Ludhiana, were highly stunted with deformed stems and leaves and failed to produce runners. On examination these plants were found infected with a bud and leaf nematode of the genus *Aphelenchoides*. Numerous individuals (males, females and other larval stages) of this nematode were extracted from stem, leaves and buds of the infected plants when immersed in water for a day. These individuals were processed and identified as *Aphelenchoides fragariae* (Ritzema Bos, 1891) Christie, 1932, the causal agent of 'spring dwarf' or 'crimp' disease of strawberry.

Some leaves of the infected plants in Gorella, Bangalore, and Dil-pasand, were having red colouration; such symptoms have also been reported to occur in this disease from Europe (Throne, 1961). Detailed studies on production of symptoms by this nematode on different varieties and other aspects are in progress.

This was first recorded from U.S.A. by Ritzema Bos (1890). Later on it has also been found to occur in some European countries. This is the first time its incidence has been recorded from India.

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---

1. Ritzema Bos, J., *Maandblad Naluurweenschappen*, 1890, **16** (7), 107.
2. Thorne, G., *Principles of Nematology*. McGraw-Hill Book Company, Inc. New York, 1961. p. 553,

INCIDENCE OF A VIRUS DISEASE OF MUSKMELON (*CUCUMIS MELO* L. VAR. *RETICULATUS*) IN W. BENGAL

ONE of the major limitations in the large-scale introduction of the improved varieties of muskmelons in W. Bengal is their susceptibility to virus diseases. The natural incidence of virus diseases of local muskmelons is surveyed in the muskmelon fields in the riverbed of the Ganges around the districts of Nadia and 24 Parganas. Three locations were selected and in each location three field plots were selected at random. In each, 100 plants were observed for the incidence of virus diseases at the end of the growing season. In this way about 900 plants ($2\frac{1}{2}$–$3\frac{1}{2}$ months old) were observed. The percentage of infected plants varied between 20 and 45. The infected plants had pale yellow leaves with green veinbanding. There was an inward curling of the leaves. Yellowish chlorotic mottle was developed from the margin of the leaves. The leaves were reduced in size and the growth of the plant also stunted. With infective sap obtained from the diseased leaves in 0·1 M citrate phosphate buffer (pH 7·2, 1 gram leaf : 1 millilitre buffer), the symptoms were easily transmitted to healthy muskmelon seedlings. The percentage of positive transmission was about 73. If phosphate buffer were used as the extraction media, the percentages of transmission were low in comparison to that obtained with material in citrate phosphate buffer.

Thermal inactivation point, dilution end point and the ageing (*in vitro*) of this virus in sap are 50° C–55° C, 1 : 50–1 : 100 and 120–144 h at room temperature and 168–192 h at 6° C respectively. The virus disease could be transmitted to cucumber (*Cucumis sativa* L. local), pumpkin (*Cucurbita moschata* Poir), rib gourd (*Luffa acutangula* Roxb.), cowpea (*Vigna sinensis* L.) and *Chenopodium amaranticolor* L. But it does not infect tobacco (*Nicotiana tabacum* L.) and Datura (*Datura stramonium* L.). In *C. amaranticolor*, it produces necrotic local lesions at the beginning of infection, but later, systemic symptoms develop.

The present study indicates that the propertie of this virus does not resemble any that has been described for cucurbit virus strains so far reported from India[1]. They however agree with that of the virus (Webb and Bohn[5]; Webb[4]; Nelson *et al.*[2]). The two viruses have similar thermal inactivation point and dilution end point. Both infect cucurbitaceous hosts or can produce loca lesions in *C. amaranticolor*. However, the ageing (*in vitro*) property of the virus, under consideration differs somewhat from that of the cucurbit latent virus, the ageing *in vitro* of which is 3 days. It is suggsted that the present virus which normally occurs in the local muskmelons, grown in the riverbeds of the Ganges, may be a strain of *Cucurbit latent virus*.

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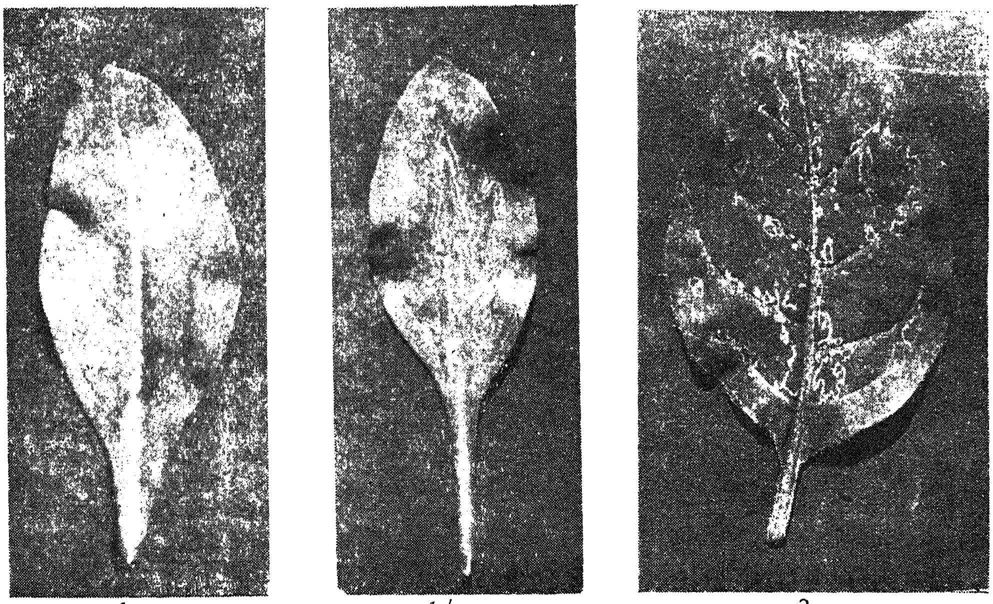
---

1. Mukhopadhyay, S., In *Current Trends in Plant Pathology*, Eds. S. P. Raychaudhuri and J. P. Verma, Lucknow University, 1974.
2. Nelson, M. R., Laborde, J. A. and McDonand, H. H., *Pl. Dis. Reptr.*, 1966, 50, 947.
3. Noordam, D., In : *Identification of Plant Viruses, Methods and Experiments*, Centre for Agricultural Publishing and Documentation, Wageningen, 1973.
4. Webb, R. E., *Pl. Dis. Reptr.*, 1964, 48, 321.
5. — and Bohn, G. W., *Ibid.*, 1961, 45, 677.

PETUNIA RINGSPOT VIRUS DISEASE—A NEW RECORD FOR INDIA

PETUNIA plants (*Petunia axillaris* and *P. violacea*) grown in Horticlture Department and University garden were observed with ringspot disease during 1976-77. This disease was characterized by the symptoms of ring formation or oak-leaf pattern (Fig. 1 *a*, *b*) on the leaves and calyx. No flower colour breaking or other ill-effects of this disease were observed. The present paper reports the transmission studies and host range of petunia ringspot disease.

For mechanical transmission, the symptomatic leaves of petunia were triturated in a pestle and mortar with cold 0·1 M phosphate buffer (pH 7·5) *plus* 0·1% thioglycollic acid and the sap was clarified at 5000 rpm in a refrigerated centrifuge for 15 minutes. The clarified sap was incoculated on the leaves of petunia and test plants, *viz.*, white Burley tobacco, *Nicotiana glutinosa*, *N. rustica*, *Chenopodium amaranticolor*, broad bean, contender cultivar of French bean and C-152 cultivar of cowpea plants which were previously dusted with 800 mesh carborundum by cotton swab method. The symptoms of rings or oak leaf pattern were produced on petunia after about one week of inoculation of infective sap. This indicated the viral nature of the disease. The symptoms on white Burley tobacco and *N. rustica* were of ringspot type (Fig. 2). However, no symptoms were produced on French bean and cowpea. The broad bean host reacted with systemic symptoms of mosaic mottling, stem streaking and wilting and *C. amaranticolor* reacted with ringspot symptoms to the virus causing ringspot disease in petunia. Petunia ringspot was also transmitted. by approach graft and by dodder (*Cuscuta reflexa* from diseased to healthy petunia plants in glass hous-


1 *a* 1 *b* 2

FIGS. 1 *a*, 1 *b*–2. Fig. 1 *a*. Petunia leaf displaying ringspot symptoms after the infection of petunia ringspot virus. Fig. 1 *b*. Petunia leaf displaying oakleaf pattern symptoms induced by petunia ringspot virus. Fig. 2. White Burley tobacco leaf displaying the symptoms of petunia ringspot virus.

For seed transmission, seeds taken from petunia plants infected with ringspot were tested in wooden flats by *growing on* test. Of the 850 seeds, about 600 seeds were germinated. However, none displayed the symptoms of ringspot disease. This indicated negative seed transmission of petunia ringspot virus through petunia true seeds.

For aphid transmission, the cotton aphids (*Aphis gossypii*), raised from single aphid colony were starved for half an hour and when fed on infected petunia leaves for fifty to sixty seconds, transmitted the disease to heatlhy petunia plants. This indicated the transmission of petunia ringspot virus by cotton aphid in non-persistent manner.

The disease, *viz.*, petunia asteroid mosaic virus, Lovisolo[3], a member of tombus virus group, Harrison *et al.*[2]; petunia ringspot virus, Rubio Huertos[5] and petunia witches' broom disease, Sahambi[6]; Mali and Vyanjane[4] have been reported so far on petunia. The present virus disease resembles petunia ringspot virus disease reported from Spain, Rubio-Heurtos,[5] in symptomatology on petunia, host plant reactions and transmission studies and in not being transmitted through the true seeds of petunia. Recently, the petunia ringspot virus has been reported as a strain of broad bean wilt virus and member of comovirus group by Doel[1] and Harrison *et al.*[2] respectively. Therefore, the present petunia ringspot virus disease is identified as a petunia ringspot virus strain of broad bean wilt virus (BBWV). This is a first record on the occurrence of petunia ringspot virus outside Spain.

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1. Doel, T. R., *J. Gen. Virol.*, 1975, **26**, 95.
2. Harrison, B. D., Finch, J. T., Gibbs, A. J., Hollings, M., Shepherd, R. T., Valenta, V. and Wetter, C., *Virology*, 1971, **45**, 356.
3. Lovisolo, O., *Boll. Staz. Pat. Veg. Roma.*, *III*, 1957, **14**, 103.
4. Mali, V. R. and Vyanjane, N. T., *Magazine, College of Agri.*, Parbhani, 1977 (In press).
5. Rubio-Huertos, M., *Microbiol. Espanola*, 1959, **12**. 325.
6. Sahambi, H. S., *Plant Dis. Problems*, *Publ. IPS*, New Delhi, 1970, p. 340.

OCCURRENCE OF *PARASARCOPHAGA MISERA* (WALKER) AND *CAMPOLETIS* SP. AS PARASITES OF *SPODOPTERA LITURA* (FABRICIUS) FROM INDIA

THE tobacco caterpillar, *Spodoptera litura* (Fabr.) (Noctuidae : Lepidoptera), is an important polyphagous pest and has become a serious pest of cauliflower crop in the Punjab. During the course of our survey, the caterpillars of *S. litura* were collected in large numbers from castor (*Ricinus communis* L.) as well as cauliflower (*Brassica oleracea* L. var. *botrytis* L.) plants in Ludhiana district and reared on fresh host leaves (Temp. 21–27·5° C; R.H.—64%) till they pupated and finally emerged as adults. Some sick and abnormal caterpillars pupated normally but later on changed to jet black in comparison with the light brown colour of the healthy pupae. This change of colour coincided with the emergence of parasitic adult flies, *Parasarcophaga misera* (Walker) (Sarcophagidae : Diptera) from the host pupae. Although *S. litura* was collected from the field from March to November but the parasites emerged only from the pupae developed from caterpillars that were collected during October and November. The percentage of parasitisation was 15 ($n = 240$) in October and 4 ($n = 75$) in November.

From a batch of 50 second and third instar caterpillars of *S. litura* collected from a cauliflower field, situated in the village Jassian in Ludhiana district, two caterpillars exhibited a very little feeding, wrinkled and shrivelled larval bodies. Such caterpillars were suspected for incipient parasitisation. Within a week, yellowish, white grubs of the suspected internal parasite forced their way out of the host sterna and pupated in the whitish cocoons which they had spun around them within few hours of their emergence. Two adult parasitic wasps of *Campoletis* sp. (Ichneumonidae : Hymenoptera) emerged out through the rounded ends of cocoons within 5 and 7 days in the laboratory.

The parasites attacking *S. litura* in India and abroad, known so far, include seven braconids, seven ichneumonids, five tachinids, two eulophids and one each of perilampid and scelionid insects. Among ichneumonids, *Campoletis chlorideae* Uchida—a larval parasite of *Heliothis armigera* Hübn., wrongly identified as *Echoropsis perdistinctus* Vier.[4] (Sudha Nagarkatti, 1977—Personal communication) is abundantly met with, around Pipariya (Madhya Pradesh) during December and January but the parasite, *Campoletis* sp. bred from the larvae of *S. litura* in the present studies was different from other known species under this genus including *C. chlorideae*. Hence the present finding of *Campoletis* sp. as a larval parasite of *S. litura* appears to be the first record. So far, *P. misera* has been reared only from *Amsacta albistriga* Walk. and *Planispira vittata* at Ooty and Coimbatore (Tamil Nadu) respectively (Sudha Nagarkatti 1977—Personal communication). Thus the present report of *P. misera* as a larval–pupal parasite of *S. litura* constitutes a new record from India.

The polyhedral inclusion bodies of a nuclear polyhedrosis virus[1] attacking *S. litura* (SLNPV) in the nature, were also evaluated against this local parasite, *P. misera,* with a view to developing this virus as microbial insecticide in future for the control of *S. litura.* The polyhedral inclusion bodies of this virus produced from experimentally infected caterpillars of *S. litura,* were inoculated to the dull-whitish grubs of *P. misera* collected by dissecting out, the field collected parasitized larvae of *S. litura* in two batches. Five grubs were dipped into the extract for 24 hours in the first batch while the second batch of 5 grubs received intrahemocoelic injections of the extract with the use of a 1 ml tuberculin syringe. Eight grubs in two groups (each of 4), treated with sterile distilled water, formed the control. It was interesting to note that all the grubs of the parasite thus treated (including control) subsequently pupated and emerged as normal adult flies from their reddish-brown puparia. These observations indicated that the viral agent was safe to the parasite. The intramuscular and intravenous injections of 44 mg of polyhedral inclusion bodies of NPV of *S. litura* at weekly intervals, could not produce any abnormality in the treated rabbits within 6 weeks[3]. Safety of similar insect viruses has also been reported earlier[2] in fishes, white mice, guinea pigs and other mammals including man. The present observations, being reported for the first time, indicate the high specificity and safety of the polyhedral inclusion bodies (SLNPV) against *P. misera.*

Thanks are due to Dr. A. S. Sidhu, Professor and Head of the Department of Entomology, for providing the facilities, and to the Director, Zoological Survey of India, Calcutta, for the identification of the parasites. The author is highly indebted to Dr. (Mrs.) Sudha Nagarkatti, Entomologist for going through the records of insect parasites being maintained at Indian Station of Commonwealth Institute of Biological Control, Bangalore.

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---

1. Battu, G. S., Dilawari, V. K. and Bindra, O. S., *Punjab Vegetable Grower,* 1976, 11, 25.
2. Burges, H. D. and Hussey, N. W., *Microbial Control of Insects and Mites,* Academic Press, London, 1971, pp. 861.
3. Ramakrishnan, N., "Nuclear polyhedrosis of *Spodoptera litura* (Fabricius)," A Lecture delivered at the Summer Institute on Insect Pathology in Relation to Biological Pest Control, I.C.A.R. and T.N.A.U., Coimbatore, June, 1976 (Unpublished).
4. Rao, V. P., *Pans,* 1968, 14, 367.

ANTIMITOTIC ACTIVITY OF THE FLAVONOL GLYCOSIDE "POLYGALACIN"

The importance of glycosides in medicine and therapeutics was indicated earlier[1]. Crude drugs containing saponin are known to exhibit antiulcerogenic and antiinflammatory actions[2]. The biochemical action of Ginseng Saponin and its metabolic stimulation or hormone-like action has also been established[3]. Some glycosides are shown to possess anticancer[4] and antimitotic[1,5-12] properties. Despite these reports there are several others whose effects on cells in division have not been investigated. The toxic principle of a flavonoid derivative (4, 5, 6, 7, 8 pentamethyloxy flavone) on Zebra fish and pregnant rats has been described[13,14]. The present study deals with the influence of the flavonol glycoside, polygalacin on mitosis in root meristems of *Allium cepa.*

Polygala chinensis L. belonging to the family Polygalaceae is a common herb found all over India. The new flavonol glycoside polygalacin ($C_{29}H_{34}O_{16}$) extracted from it is shown to be 3-rutinosyl rhamnazin (3-rutinosyl 7, 3-dimethyl quercetin)[15]. Profusely growing roots of *A. cepa* were treated in one series in 0·05% polygalacin (aqueous solution prepared by gently heating) for 4, 8 and 24 hr at room temperature. In another, roots treated for 24 hr were allowed to recover for 24 and 48 hr in distilled water with frequent changes of the same. Controls grown in distilled water were handled under identical conditions. Experimental and control roots fixed in 1 : 3 acetic alcohol were processed by the method described elsewhere[1,16].

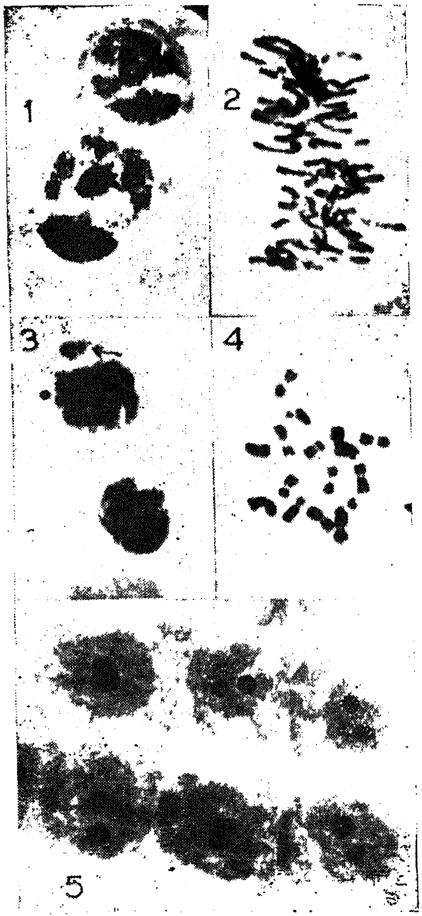
The most obvious effects produced by the glycoside were on the morphological changes of chromosomes and a lowering of mitotic index (MI). The MI showed a phenomenal decrease with the period of treatment (Table I). In 24 hr material mitoses were, however, highly limited. There was also a marked effect on cells in producing fragmented nuclei (Fig. 1). The latter were found even after a protracted period of treatment where mitoses were still frequent. Among the aberrations the breakage of chromosomes (Fig. 2) was an important event noted in 8 and 24 hr treatments. The micronuclei obviously formed from fragments

TABLE I

Treatment time (in hours)	Cells in division*	Mitotic index*	Frequency of aberrations				
			Fragmented nuclei	Breakages	C-metaphases	Micronuclei	Abnormal cells (%)
Control	264	6·60	..	..	..	..	..
4	232	5·80	21	..	..	..	9·05
8	208	5·20	44	8	5	..	27·40
24	128	3·20	67	14	9	10	78·12
24 hr + 24 hr recovery	224	5·60	..	..	..	..	..
24 hr + 48 hr recovery	260	6·50		..	..	..	..

* Based on 4,000 cells.

were encountered in 24 hr treatment only (Fig. 3). Slightly disturbed but unscattered and uncontracted metaphases were seen in 4 hr treatments. On the other hand 8 and 24 hr material exhibited few C-metaphases thus indicating the disruption of the spindle (Fig. 4). In contrast to another glycoside Morindine reported elsewhere[1] C-metaphases induced by polygalacin were few and occasional. One more striking observation was that the nuclei were prominently stained in majority of cells in 8 and 24 hr treatments in contrast to faint nuclear staining (Fig. 5). This appears to be due to the specific response of the cells to polygalacin alone since no such feature was demonstrated by Morindine[1]. The mechanism by which the phenomenon is induced needs further investigation.



FIGS. 1–5. Polygalacin induced abnormalities in *A. cepa*. Haematoxylin squashes. Fig. 1. Nuclear fragmentation, 8 hr × *ca.* 1,000. Fig. 2. Chromosome breakage, 24 hr × *ca.* 1,000. Fig. 3. Micronucleus (arrow), 24 hr × *ca.* 1,000. Fig. 4. C-metaphase, 24 hr × *ca.* 1,000. Fig. 5. Nucleolar staining, 24 hr × *ca.* 1,000.

Morindine and polygalacin have antimitotic properties. While the former showed a pronounced induction of C-metaphases, stickiness of chromosomes and a slow recovery[1], the latter produced nuclear fragmentation, occasional C-metaphases, breakages and a fairly quick recovery. The return of the mitotic index, in roots recovered for 24–48 hr, to the levels of short period treatment and the control and an absence of abnormal cells in such material (Table I) denotes the transient nature of the damage caused by polygalacin to the genetic system and its reversibility.

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enkat Reddy, P., Rao, P. S. and Subramanyam, S., *Curr. Sci.*, 1976, 45, 528.
amahara, I., Shintani, Y., Kono-hima, T., Sawada, T. and Fujimura, H. Yakugaku Zasshi, *Jour. Pharm. Soc. Japan*, 1975, 95, 1179.
ura, H., Hirai, S., Oklaka, Y. and Yokozawa, T., *Jour. Biochem. (Tokyo)*, 1975, 77, 1057.
ylor, A., McKenna, G. F. and Burlage, H. M., *Texas Reports of Biology and Medicine*, 1956, 14, 538.
enkateswarlu, J. and Srinivasan, K., *Mem. Ind. Bot. Soc.*, 1960, 3, 78.
lah, I. A., *Bull. Torrey Bot. Club*, 1965, 92, 197.
upchan, S. M., *Jour. Med. Chem.*, 1969, 12, 167 (Cited by Oświecimska *et al.*, 1975).
ukowska, L. A., *Hereditas*, 1971, 67, 205.
, *Acta Soc. Bot. Pol.*, 1971, 40, 623.
eller, L., Kuhn, C. M., Von Wartburg, A. and Stachelin, H., *Jour. Med. Chem.*, 1971, 14, 936.
n, W. C., Kupelman, M., Wilson, R. A. and Rao, K. V., *Phytochem.*, 1972, 11, 172.
wiecimska, M., Sendra, J. and Janeczko, J., *Pol. Jour. Pharmacol. Pharm.*, 1975, 27, 349.
ne., R. W., Stout, M. G., Reich, H. and Huffman, M. N., *Cancer Chemotherapy Rep.*, 1964, 34, 19.
out, M. G., Reich, H. and Huffman, M. N., *Ibid.*, 1964, 36, 23.
ahalakshmi, I. V., Rao, P. S. and Zitendranath, P., *Ind. Jour. Chem.*, 1976, (Communicated).
bramanyam, S. and Subramanian, M. K., *Proc. Ind. Acad. Sci.*, 1970, 72 B, 115.

t consulted in original

OXIPHIUM : A NEW GENERIC RECORD FROM INDIA

the course of surveys on fungal diseases iosperms in Banaras Hindu University a sooty mould was prevalent, on *Ficus* L., *Mangifera indica* L., *Hibiscus rosa-* L., *Tectona grandis* L. and *Gossypium* *m* L. The fungus is a new generic record causing deterioration of plants.

ungus formed a thick network of hyphae, doparanchymatous crust on the leaves and the smaller twigs involving the entire tem.

ungus was identified as *Microxiphium fagi* n. Huges, colonies effuse, brownish black, ct synnematous conidiophores. Synnemata 15 μ long, 28 μ 55 thick at the base. nemata capped by a slimy head. Annellides al, 8-12 μ × 1-2 μ. Conidia aggregated in slimy head, acrogenous, one celled, ovoid, smooth, hyaline, 3-4 μ long (Figs. 2, 3).

FIGS. 1-3

Microxiphium was first used at the generic level by Thumen[1] (1879). The specimen has been deposited in C.M.I., herb. Nos. 208617 and 208629.

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---

1. Thumen, F. de, *Mycotheca Universalis, Centuria*, Wein, 1879, 14, 1352.
2. Huges, J. S., *Mycologia*, 1976, **68**, 693.

DIFFERENTIAL RESPONSE OF *RHIZOBIUM* STRAINS OF BENGAL GRAM (*CICER ARIETINUM* L.)

DIFFERENT strains of *Rhizobium* sp. interact differently with different genotypes of Bengal gram[1]. Sundra Rao and Sen[7] reported an increase of 17–31% in grain yield due to *Rhizobium* inoculation in Bengal gram. The present investigation was carried out in order to evaluate the efficiency of the different *Rhizobium* strains with genotype H 208 of Bengal gram in calcarious soil of North Bihar.

Eight *Rhizobium* strains obtained from I.A.R.I., New Delhi, *viz.*, G.F. 2, G.F. 3, G.F. 5, G.F. 6, G.F. 7, G.F. 8, G.F. 9 and G.E. 8 were selected for this study. The field experiment was conducted at Agricultural Research Institute, Dholi (Bihar) in a randomised block design with three replications, the net plot size being 2 × 0·9 m. The soil of the experimental plot was sandy loam, having pH 8·6, $CaCO_3$ 37·8%, E.C. 0·416 mmhos/cm, Organic carbon 0·456% and available P_2O_5 41 Kg/ha.

The seeds after sterlization were slurry inoculated with different strains in 10% sugar solution and sown in the field. The survival of *Rhizobium* o different seeds was examined and more than 1 cells per seed were found in all cases. Fertiliz at 20 Kg N and 50 Kg P_2O_5 per hectare were appli A uniform plant population was maintained. Th plants were dug up carefully, washed in slow runni water and the nodulation examined.

All the strains proved significantly superior control in respect of grain yield. Among the vari strains, G.F. 9, G.F. 2 and G.F. 8 were fou equally efficient in recording higher grain yi than the other remaining strains. Per cent increa in yield due to different *Rhizobium* strains over co trol was in the range of 13·9 to 39·8%. Th highest increase in yield (39·8%) was record with strain G.F.9 (Table I).

TABLE I

Effect of different Rhizobium *strains on grain yield, number of nodules and dry weight of nodules per plant*

Treatments	Grain yield in kg/ha	No. of root nodules per plant	Dry weight of root nodules per plant in mg	Per cent increase in grain yield over control
G.F. 2	1452	5·5	122	27·6
G.F. 3	1508	3·5	142	32·5
G.F. 5	1307	4·2	127	13·9
G.F. 6	1471	2·0	145	28·9
G.F. 7	1434	4·3	103	26·0
G.F. 8	1443	4·2	160	26·9
G.F. 9	1591	3·7	150	39·8
G.E. 8	1314	3·8	133	15·4
Control (uninoculation)	1138	2·5	137	..
C.D. at 5%	150	..	..	..

The difference in production of root nodules a their dry weight/plant due to strains were no significant indicating thereby no correlation b between grain yield and number or their dry weig This may be accounted for differences in nitro fixing capacity of the strains due to their gene character. Moreover, the yield is governed by t amount of nitrogen fixed rather than the numb or dry weight of nodules as nitrogen fixation positively correlated to the leghaemoglobin conte of nodules which is also genetically controlle Genetic information for nitrogenase is encod in *Rhizobium* and expression of nitrogenase gene depends on unknown diffusible factor(s) secret by plant' cells[5,3,4]. The present investifat supports the genes coding for nitrogenase *Rhizobium* strains. The demonstration of nitr enase in presence of legumes suggested that some ommon plant product may be involved[6,2].

The authors thank Dr. R. B. Rewari, Micro-iologist, I.A.R.I., New Delhi, for providing hizobium strains. Thanks are also due to r. R. Singh, Principal and Regional Director, irhut College of Agriculture and Agricultural esearch Institute, Dholi, for providing necessary cilities.

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ihar, *April* 25, 1977.

---

1. Bapat, P. N. and Vaishy, U. K., *Report of Microbiological Work on Pulses Conducted During Rabi Season of* 1975–76, J.N.K. Vishwa Vidyalaya, Jabalpur (M.P.).
2. Child, J. J., "Nitrogen fixation by *Rhizobium* sp. in association with non-legumes plant cell cultures," *Nature*, 1975, 253, 251.
3. Dilworth, M. J. and Parker, C. A., *J. Theoret. Biol.*, 1969, 25, 208.
4. Dunican, L. K. and Tierney, A. B., *Biochem. Biophys. Res. Commun.*, 1974, 57, 62.
5. Phillips, D. A., Howard, R. L. and Evans, H. J., *Physiol. Plant*, 1973, 28, 248.
6. Scowcroft, W. R. and Gibson, A. H., *Nature*, 1975, 253, 250.
7. Sundra Rao, W. V. B. and Sen, A. N., *Rhizobium News Letter*, 1969, 41, 35.
8. Vincent, J. M., *The Biology of Nitrogen Fixation*, Edited by A. Guispel, 1974, 33, 263.

UCLEIC ACID CHANGES IN PETUNIA ROOTS AS A RESULT OF PETUNIA MOTTLE VIRUS INFECTION

the present communication the result of the ffect of petunia mottle virus (PMV) on the nucleic cid content in the roots of petunia has been reported.

Seedlings of petunia raised in autoclaved soil, ansplanted to clay pots containing autoclaved mixture f soil : sand : compost (7 : 2 : 1). Leaves of two-eek old seedlings were inoculated with (PMV) at 0, 20, 30 and 40 days intervals separately. For oculation, the usual method of rubbing with carbo-ndum (500 mesh) as an abrasive was adopted. The xperiment was carried out in an insect-proof glass ouse and it was terminated after 10 days of last oculation. Plants were uprooted, washed and ried (80–90° C) and were ground into a fine powder. undred mg of the powder were extracted as described y Srivastava and Ware[1] and the nucleic acids estimated y the method of Scheneider[2] employing Buach and omb Spectronic-20 Spectrometer, at 660 nm using NA and DNA as standards.

It may be observed from Table I, that there was an increase in the RNA content inside the plant roots as compared with the uninoculated control; however, the maximum (81·2%) increase was noted in the plants inoculated 10 days before the harvest and least (37·3%) in the plants inoculated 40 days before harvest. Though the DNA content also showed an increase in the roots as compared to uninoculated (control), maximum increase (42·6%) was observed in plants inoculated 40 days before harvest and minimum increase (5·7%) in the plants inoculated 10 days before harvest.

TABLE I

Nucleic acid contents in the roots of petunia inoculated with Petunia Mottle Virus at different intervals

(Expressed as per cent dry weight)

Nucleic Acids	Inoculation periods (days)				
	0	10	20	30	40
RNA	3·21	5·81	4·60	4·41	4·41
DNA	0·35	0·37	0·40	0·45	0·50
Total	3·56	6·18	5·00	4·86	4·91

Each value is the mean of 6 replicates.

The authors are grateful to Dr. M. M. R. K. Afridi for his valuable suggestions and to Prof. Abrar M. Khan, Head, Department of Botany, for providing necessary facilities.

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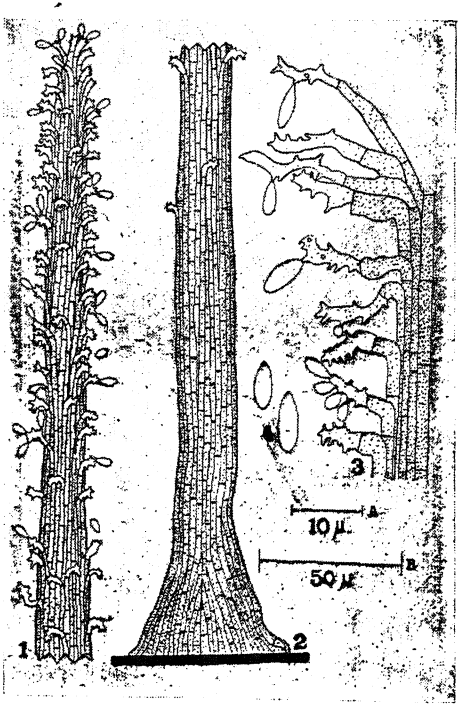
1. Srivastava, B. I. S. and Ware, G., *Plant Physiol.*, 1965, 40, 62.
2. Scheneider, W. C., *Methods in Enzymology—III* (Eds. S. P. Clowick and N. O. Kapan), Acad. Press, New York, 1957.

PHAEOISARIA CLEMATIDIS (FUCKEL) HUGHES[1], A NEW RECORD FOR INDIA

IN the course of their studies on mycoflora of District Nimar, the authors collected *Phaeoisaria clematidis*, a dematiaceous hyphomycete on dead twigs of *Bambusa* species, from Asirgarh, Khandwa (M.P.), on 18th October, 1976. The fungus has been previously recorded from Cuba, Europe, Ghana, Java, Malaya, New Guinea; Sabah and Sierra Leone (Ellis)[2]; here it is being presented as a new record for India.

Colonies effuse, black, hairy; under a disssecting microscope the upper two-thirds of each synnema is seen to be covered with a white or pale grey powdery mass of conidia. Mycelium mostly immersed. Stroma, hyphopodia and setae absent. Conidiophores macronematous, synnematous; synnemata brown, upto 2·0 mm*. high but usually less than 1·3 mm. subulate, 7–20 μ thick at the apex, 25–90 μ thick at the base; individual threads 2–3 μ thick, pale to mid brown, smooth, straight or flexuous, splaying out at the apex and along the sides of the two-thirds of each synnema. Conidiogenous cells polyblastic, integrated and terminal or discrete, cylindrical or clavate, hyaline to pale brown, sympodial, usually with numerous cylindrical denticles. Conidia solitary, dry, acropleurogenous, fusiform or narrowly ellipsoidal, hyaline or subhyaline, smooth, non-septate, 4–7–10 × 1·5–2·5–3 μ.



FIGS. 1–4. *Phaeoisaria clematidis*. Figs. 1–2. Upper and lower half of a synnema. Fig. 3. A part of synnema with conidiophores and conidiogenous cells. Fig. 4. Conidia. (Figs. 1–2 to scale B. Figs. 3–4 to scale A.)

The voucher specimen has been deposited in the herbarium of Mycology and Plant Pathology, Department of Botany, Government S.N. College, Khandwa, as No. 48. The authors express their grateful thanks to Dr. S. B. Saxena, University of Saugar and Dr. V. B. Chaudhary, Government S.N. College, Khandwa, for facilities.

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ANITA DAVE.

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* Measurements by Ellis: Synnemata upto 1·5 mm high, 20–80 μ thick at the base, 8–25 at the apex; conidia 4–10 × 1·5–2·5 μ.

1. Hughes, S. J., *Can. J. Bot.*, 1958, 36, 795.
2. Ellis, M. B., *Dematiaceous Hyphomycete*, C.M.I., Kew, Surrey, 1971, p. 213.

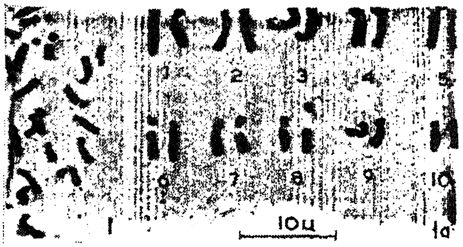
CYTOGENETICS OF AQUATIC ORNAMENTALS

III. Karyology of *Victoria amazonica* Sowerby

THE Amazon waterlily represents a remarkable form of plant life on account of its gigantic floating leave and copious development of prickles on the surfa of its organs. Taxonomists had been calling th *V. regia* for the last so many decades, but is has recently been named *V. amazonic* by Prance and Prance[1]. Cytological findings made so far on the genus *Victoria* confin only to chromosome counts[2-4]. The present no deals with a detailed study of the architecture of the somatic chromosomes of *V. amazonica* in th light of its evolutionary status and inter-relationship with the other members of Nymphaeaceae.

The Plants of *V. amazonica* were introduced i National Botanic Gardens, Lucknow, through seed which had been obtained in 1975 from Leide University Garden, Netherlands. Chromosom studies were done from healthy root-tips whic were pretreated with 0·002 M 8-hydroxyquinoli for $2\frac{1}{2}$ hr at 8°–12° C, fixed in acetic-ethanol mixtu (1 : 3) and finally squashed in aceto-orcein.

Observations, based on twenty metaphasic cell revealed a constant chromosome number $2n =$ (Fig. 1). Total chromatin length of a compleme



FIGS. 1 and 1 *a*. Photomicrograph of th somatic metaphase plate and idiogram o *V. amazonica*.

was recorded 88·92 μ, whereas lengths of th individual chromosomes varied between 3·08 μ a 5·39 μ. All the chromosomes could be classifie on the basis of their centromeric positions, into two groups, *i.e.*, four pairs of sub-metacentric and six pairs of sub-acrocentric types (Fig. 1 *a*). Of these, one pair of medium sized sub-acrocentric chromosomes possessed secondary constrictions.

Present finding of 20 chromosomes in this species confirms an earlier report of Langlet and Soderberg[2]. The chromosomes are characterised by considerably long sizes with more or less symmetrical karyotype. Such long chromosomes have not been reported in other genera of Nymphaeaceae except *Nelumbo*, where the karyotype appears highly asymmetrical[5]. According to Darlington[6] and Stebbins[7] the symmetrical karyotype with long metacentric to acrocentric chromosomes indicate primitiveness of the genus whereas telocentric type and/or abrupt size difference of chromosomes in a complement represent evolutionary advance. Keeping this view in mind, *V. amazonica* can be considered primitive on the basis of its chromosome number and details of the somatic complement to the other existing members of Nymphaeaceae. The morphology of this taxon also is reflective of its primitiveness and supports the cytological data.

The encouragement and facilities given by Dr. T. N. Khoshoo, Director of National Botanic Gardens, are acknowledged.

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April 8, 1977.

---

1. Prance, G. T. and Prance, A. E., *Garden Journal*, 1976, 26 (4), 118.
2. Langlet, O. F. J. and Soderberg, E., *Acta Hort. Berg.*, 1927, 2, 85.
3. Heitz, E., *Planta*, 1932, 18, 571.
4. Harada, I., *Jap. Jour. Genet.*, 1952; 27, 117.
5. Gupta, P. P., *Cytologia*; 1976, 41, 761.
6. Darlington, C. D., *Chromosome Botany and the Origins of Cultivated Plants*, Hafner Publishing Co., New York, 1963.
7. Stebbins, G. L., *Chromosomal Evolution in Higher Plants*, Edward Arnold Ltd., London, 1971.

IMPROVEMENT OF YIELD, CARBOHYDRATE AND NITROGEN LEVELS IN CORN BY ALACHLOR

CERTAIN triazine herbicides are known to enhance yield at sublethal concentrations. Increased yield is also associated with increased protein and nitrogen levels[1]. Studies on the effect of chemical herbicides on stomatal behaviour indicated the possible use of certain amide group herbicides as antitranspirants[2,3]. Subsequently, alachlor, an amide type herbicide has been tried in this laboratory on maize plants as an antitranspirant. The results obtained with alachlor were compared with a known chemical antitranspirant, PMA[4]. We have already reported that alachlor can be used as an ideal antitranspirant by reducing the water loss without adverse effects (communicated) The increase in yield by triazine type herbicides was associated with increase in protein and nitrogen levels without affecting its nutritive value[1]. The principal aim of this investigation therefore has been to study the influence of alachlor on crop yield and nutritive value of maize seeds.

Seeds of maize (*Zea mays* L. var. Hi-starch) were sown at 1 cm depth in experimental plots of 10 × 4 M of loam soil mixed with farm-yard manure in 3:1 proportion. Soil moisture levels were maintained at 20 per cent level by regulating watering. When plants were two-week-old the chemicals, phenyl mercuric acetate at 15 mg/l and alachlor [2-chloro-2, 5-diethyl-N-(methoxymethyl) acetanilide] at 20 mg/l were sparyed on foliage without any wetting agent. A second spray was given thirty days after the first treatment.

The crop was harvested after a period of 80 days. Yield was expressed in terms of 1000 seed weight, length and diameter of the cobs and also in dry weight of the stem. Harvest index was also determined as per the definitions of Donald[5]. Nutritive value of the seed was determined by estimating the protein and nitrogen fractions according to the methods of Markham[6] and Thimann & Loos[7]. Carbohydrate fractions were determined by the method of Nelson[8]. Starch content was estimated according to the method of McCready *et al.*[9].

Effects of alachlor (20 mg/l) and PMA (15 mg/l) on yield parameters of maize plants are shown in Table I. An increase in the yield with alachlor treatment and a decrease with PMA as compared to the controls are noticed. Associated with the increase in yield, cob height and diameters are also higher in alachlor treated plants over control. Seed shape and size are also decreased with PMA, while they are higher than controls with alachlor (Table I).

Influence of alachlor and PMA on carbohydrate levels of seeds is shown in table II. Reducing and non-reducing sugars are increased with both alachlor and PMA treatments. But the increase is more prominent in PMA treated than in alachlor treatment. The starch content is enhanced with alachlor treatment while it decreases with PMA treatment. Similarly, the levels of protein nitrogen, soluble nitrogen and total nitrogen also increased over the controls under alachlor treatment while they decreased with PMA (Table II).

Simazine and atrazine were already known to increase the protein content in certain crops[10]. Tweedy and Ries[11] found that the addition of simazine to corn plants increased not only the total nitrogen and dry

TABLE I

*Effect of alachlor or PMA on yield parameters of maize plants**

Treatment	** Average height of cob (cm)	** Average diameter of cob (cm)	*** Straw weight 100 plants (Kg)	*** 1000 Seed weight (g)	No. of seeds/ cob	Harvest index ratio	Economic production as % of biological production
Control	10·70 (100)	9·7 (100)	30·0 (100)	220·60 (100)	442 (100)	0·40	26·5%
Alachlor 20 mg/l	13·65 (131·30)	10·3 (106·2)	37·5 (125·0)	270·27 (122·70)	560 (126·6)	0·51	32%
PMA 15 mg/l	8·05 (75·23)	8·59 (88·7)	25·0 (83·33)	160·25 (72·72)	264 (59·4)	0·24	15%

* Mean of five individual experiments.
** Average of 60 observations.
*** Average of ten observations.
Figures in parenthesis represent the % of control.

TABLE II

*Influence of alachlor or PMA on carbohydrate and nitrogen levels of maize plants**
(mg/g dry weight of seed)

	Control	Alachlor 20mg/l	PMA 15 mg/l
Reducing sugars	10·54	13·72	16·60
Non-reducing sugars	10·21	11·28	23·69
Starch	438·50	482·79	402·24
Protein nitrogen	65·10	104·16	56·42
Soluble nitrogen	54·66	87·36	47·32
Total nitrogen	119·71	191·52	103·74

* Mean of five individual experiments.

weight of the plants but also the activity of nitrate reductase. Singh, Salunkhe and Lipton[12] observed that the herbicide of the S-triazine type increased the protein, soluble protein and amino acids but decreased the starch and total sugars in the leaves of bush bean plants. Singh and Salunkhe[13] suggested that the increase in protein content associated with S-triazines may be due to an increased rate of amino acid formation resulting from the higher rates of both NRase and transaminase activities. Similarly we have noticed the enhanced yield associated with the increased protein and nitrogen levels in maize seeds.

From the present study, it is believed that alachlor can be used to inrcease the crop yield and seed nutritive value of corn and its effectiveness to improve crop productivity in dry land areas should further be explored.

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1. Ries, S. K., Moreno, O., Meggitt, W. F., Schweizer, C. J. and Askhar, S. A., *Argon, J.*, 1970, **62**, 746.
2. Das, V. S. R. and Santakumari, M., *Proc. Ind. Acad. Sci.*, 1975, **82**, 108.
3. Santakumari, M., *Ph.D. Thesis*, Sri Venkateswara University, Tirupati. 1975.
4. Gale, J. and Hagen, R. M., *Annu. Rev. Plant Physiol.*, 1966, **17**, 269.
5. Donald, C. M., *J. Aust. Inst. Agric. Sci.*, 1962, **28**, 171.
6. Markham, R., *Biochem., J.*, 1942, **36**, 790.
7. Thimann, K. V. and Loos, G. M., *Plant Physiol.*, 1957, **32**, 274.
8. Nelson, N., *J. Biol. Chem.*, 1944, **153**, 375.
9. McCready, R. M., Guggole, J., Silviera, V. and Owens, H. S., *Anal. Chem.*, 1950, **29**, 1156.
10. Wu, M. T.. Singh, B. and Salunkhe, D. K., *Phytochemistry*, 1971, **10**, 2025.
11. Tweedy, J. A. and Ries, S. K., *Plant Physiol.*, 1967, **42**, 280.
12. Singh, B., Salunkhe, D. K. and Lipton, S. H., *Hort. Sci.*, 1972, **47**, 441.
13. — and – , *Can. J. Bot.*, 1970, **48**.

SOME ADDITIONS TO THE LICHEN FLORA OF INDIA

III. Genus *Relicina* (Hale & Kurk.) Hale (Parmeliaceae)

THE genus *Relicina,* a segregate of the composite genus *Parmelia,* has recently been monographed on world level by Hale[3] (1975), and it includes 24 species almost all of which occur in tropical south-east Asia. Hale has further mentioned that the genus is yet unknown in Europe, India, Central Asia and Africa (p. 12). We collected two species of the genus *Relicina* in Cardamom hills, Kerala and these have also not been reported in the recent monograph of the Indian species of the collective genus *Parmelia* by Awasthi[1] (1976).

In this note, we give descriptions of the two species of the genus *Relicina.* Chemical studies were carried out by thin layer chromatography (Culberson[2], 1972). Specimens referred to in the text are deposited in the Lichen Unit of the Ajarekar Mycological Herbarium (AMH).

1. *Relicina abstrusa* (Vain.) Hale, *Phytologia* 28 : 484, 1974.

   ≡ *Parmelia abstrusa* Vain. *Acta Soc. Faun. Fl. Fenn.* 7 (7) : 64, 1890.

Thallus 5–8 cm broad, pale-yellowish green, closely adnate to the substratum, corticolous or saxicolous, dissected into lobes ; lobes sublinear to linear, 1·0–1·5 mm broad, upper surface smooth, plane to slightly convex; moderately isidiate ; isidia short, simple, cylindrical ; cilia bulbate, slightly inflated ; lower surface black to the margin, rhizinate, rhizines moderately developed, simple. Apothecia not seen.

Chemistry : Cortex K + yellow ; medulla K + yellow turning red, C –, KC –, P + orange. Norstictic acid and usnic acid are present.

Specimens examined : Kerala : Cardamom hills, Munnar–Kumily Road, near Begampara—76·680, 808, 809, leg. P. G. Patwardhan.

Distribution : North America, Central America, South America, Japan, Taiwan, Philippines, Malaya, Indonesia, Sarawak and now India.

Remarks : *Relicina abstrusa* is a common species in both New as well as Old World. The New World species are reported to produce salacinic acid along with norstictic acid.

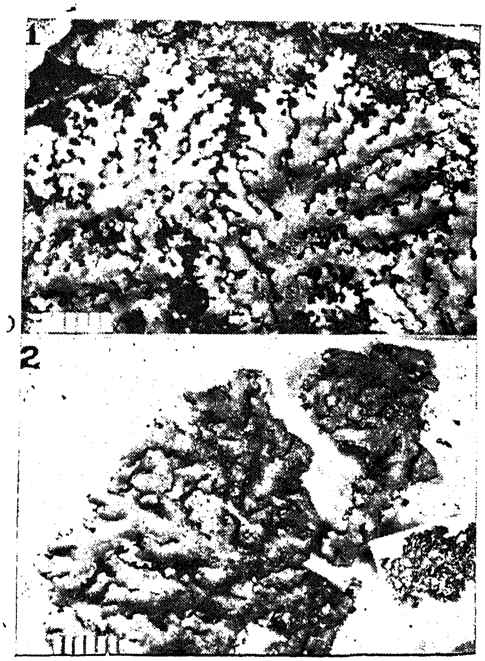
2. *Relicina sydneyensis* (Gyel.) Hale, *Phytologia* 28 : 485, 1974.

   ≡ *Parmelia sydneyensis* Gyel. *Ann. Mycol.* 36 : 292, 1938.

Thallus closely adnate to the substratum, corticolous or saxicolous, 3–5 cm broad, dark green with yellowish tinge (due to usnic acid), divided dichotomously into sublinear to linear-elongate lobes, lobes 1·0–2·0 mm broad, upper surface smooth, isidiate ; isidia simple, cylindrical ; bulbate cilia distinctly inflated ; lower surface pale brown, sparsely rhizinate, rhizines simple, mostly towards the centre. Apothecia not seen.

Chemistry : Cortex K + yellow ; medulla K + yellow turning red, C –, KC –, P + orange. Atranorine, usnic acid, norstictic acid and stictic acid are present.

Specimen examined : Kerala : Cardamom hills, Munnar–Kumily Road, near Gudampara—76.659 (Part in US) ; leg. M. E. Hale.



FIGS. 1–2. Fig. 1. *Relicina abstrusa* (Vain.) Hale. Fig. 2. *R. sydneyensis* (Gyel.) Hale—Scale in mm.

Distribution : Japan, Taiwan, Java, Australia and now India.

Remarks : *Relicina limbata* (Laurer) Hale, a non-isidiate species, is very closely related to *R. sydneyensis* which, however, is a isidiate species.

Thanks are due to Prof. M. N. Kamat, M.A.C.S. Research Institution, Poona-4, for his interest ; and Dr. Mason E. Hale, Smithsonian Institution, Washington, D.C., U.S.A., for valuable suggestions. The work was supported by a National Science Foundation, Washington, D.C., U.S.A. grant

(GF-36745) to the M.A.C.S. Research Institution, Poona-4.

Dept. of Mycology and Plant Pathology, Maharashtra Association for the Cultivation of Science, Law College Road, Poona 411 004, India, *June* 2, 1977.

P. G. PATWARDHAN.
A. V. PRABHU.

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1. Awasthi, D. D., *Biol. Mem.*, 1976, 1 (1 and 2), 155.
2. Culberson, C. F., *J. Chromatogr.*, 1972, 72, 113.
3. Hale, M. E., "A monograph of the lichen genus *Relicina* (Parmeliaceae)," *Smithsonian Contrib. Bot.*, 1975, 26, 1.

CHAETOMIUM JABALPURENSE, A NEW FUNGUS FROM SOIL

DURING the course of studies on fungal succession in the soil of grazed grassland of Jabalpur, M.P., India, a hitherto unknown species of *Chaetomium* was isolated and is herewith described.

Perithecia superficial, olive-brown to almost black, densely clothed with dark olive brown hairs, globose to subglobose with more or less rounded base, 154·7–304·5 × 150·3–297·2 μ, ostiolate attached firmly to the substratum by a few yellowish-brown rhizoids. Terminal hairs forming a dense head at the top of perithecium, brownish-black at the base, gradually becoming lighter in colour towards the apex, always unbranched, uniformly thick, 3·2 μ wide with rounded tips, apparently unseptate, coarsely roughened with minute projections, below straight or nearly so, becoming coiled anticlockwise into 6–10 regular close spirals above, coils extending above the spore mass. Lateral hairs dark olive-brown, straight below anticlockwise spirally coiled into 1–6 spirals above, apparently aseptate, minutely roughened, 3·2–3·6 μ wide. Asci clavate, hyaline, thin walled, evanescent, eight spored, 31·0–46·5 × 9·3–12·5 μ (spore bearing part 21·7–24·8 μ in length). Ascospores irregularly biseriate in the asci, dark olivaceous brown, fusiform-elliptical to rhombo-ellipsodial, 8·7–12·4 × 5·3–6·2 μ, narrowly rounded at one end or both ends, frequently produced in cirrhi.

The present isolate resembles closely to *Chaetomium spiralotrichum* Lodha[1] in having spirally coiled terminal and lateral hairs and the shape of perithecia, but differs in that the terminal and lateral hairs are always unbranched and anticlockwise coiled. The ascospores are typically fusiform to rhombo-ellipsoid as against ellipsoid ascospores found in *C. spiralotrichum.* It also resembles *Chaetomium gelasinosporum* Aue and Müller[2] in the nature of terminal hairs, but distinctly differs in having spirally coiled lateral hairs and larger perithecia, asci and ascospores which are also of different shapes.

In view of these differences, the present isolate is described as a new species and named *Chaetomium jabalpurense* after the place of occurrence.

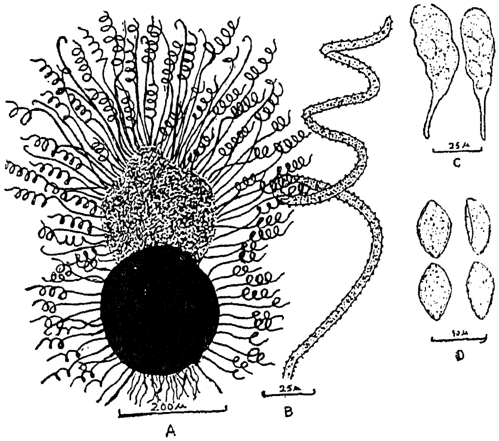


FIG. 1. *Chaetomium jabalpurense* sp. nov. A. Perithecium; B, Upper portion of terminal hair; C, Asci; D, Ascospores.

Chaetomium jabalpurense Tiwari, Agrawal and Lodh sp. nov.

Peritheciis superficialibus, olivaceo-brunneis vel prope nigris, globosis vel sub-globosis, 154·7-304·5 × 150·3–297·2 μ, ostiolatis; ad substratum cum rhizoidii pallide luteobrunneis leviter affixis Capillus terminalibus brunneolo-nigris inferne pallidiores in ultima, non-ramosis, 3·2 μ latiapicibus rotundatis, inhonspique septatis; menifeste minute granuloso incrustatis, basi rectis, spiraliter curvatis in apicis, spiris 6–10 anticlock typus extensi ultra sporarum massum. Capillus lateralibus fusce olivaceo-brunneis, inferne recti in ultima spiraliter curvatis, spiris 1–6 anticlock typus, apparenter aseptatis, leviter asperis, bas latis 3·2–3·6 μ. Asci clavatis, octosporis hyalini stipitatis, evanescentibus, 31·0–46·5 × 9·3–12·5 (parte sporas fereute longa 21·7–24·8 μ). Asco sporis inaequate biseriatis, fusce olivaceo-brunnei fusiforme ellipsoides vel rhombo-ellipsoides, 8·7 12·4 × 5·3–6·2 μ, anguste rotundatus ad utroq apicis, saepae cirrhos prolatis.

Typus lectus in solo, Jabalpur, M.P., India Cultura typica postea in C.M.I., Kew, Surrey England, No. IMI, 157256.

Colonies on potato-dextrose and potato-mal cellulose agar media growing rapidly at 28° C attaining a diameter of 48 mm. in 7 days. Aerial mycelium dull grey, thin, showing saltation zones. Reverse citrus yellow. Perithecia appear late towards margin; they are irregularly scattered and are formed singly or in clusters.

The authors are grateful to the Director, C.M.I., Kew, England and Dr. D. L. Hawksworth, for their opinion concerning *C. jabalpurense* and to the Principal, Government Science College, Jabalpur, for facilities.

Department of Botany,
Govt. Science College,
Jabalpur 482 001,
June 3, 1977.

D. P. Tiwari.
P. D. Agrawal.
A. R. Lodh.

---

1. Lodha, B. C., *J. Indian bot. Soc.*, 1964, 43, 121.
2. Aue, R. and Müller, E., *Ber. Schweiz. Bot. Ges.*, 1967, 77, 187.

CHROMOSOME NUMBER IN SOME SPECIES OF *LAGERSTROEMIA*

The species of the genus *Lagerstroemia* Linn. (family Lythraceae) on account of their prolificity of beautiful flowers are very popular ornamental trees and shrubs and form an important part of tropical-subtropical landscape. A systematic programme of breeding new types has been started at the National Botanic Gardens, Lucknow. In view of the contradictory chromosome numbers (Darlington and Wylie[1], 1955; Bolkhovskikh *et al.*[2] 1969) reported for 4 species out of the 30 reported in the genus, as a first step, critical chromosome number determinations have been made and the results are reported in this communication. Studies were based on male meiosis following the usual aceto-carmine squash method.

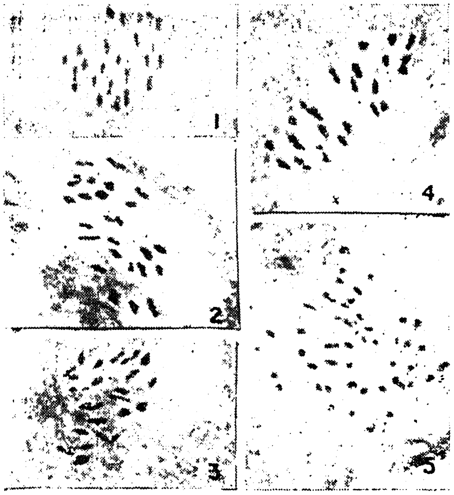
The present work together with earlier one on the genus is summarised in Table I.

At metaphase I in the pollen mother cells in all the species 24 bivalents could be clearly counted (Figs. 1–4). The genus appears to be characterised by small chromosomes. Anaphase I and further course of meiosis in the species is regular resulting in normal pollen fertility. The chromosome number in the species *L. parviflora* Roxb. and *L. thorelli* Gagnep has been reported for the first time.

Table I

Chromosome number reports in Lagerstroemia

Taxon	*n*	2 *n*	Pollen fertility (%)	Reference
L. flosreginae Retz.	..	44	..	Tjio, 1948
	..	48	..	Nanda, 1962
	24	..	89·5	Present author
L. parviflora Roxb.	24	..	66·1	Present author
L. thorelli Gagnep.	24	..	81·45	Present author
L. indica Linn.	..	50	..	Bowden, 1945
	24	..	87·1	Present author
L. × *lancesteri* Hort.	..	44, 46, 48, 64	..	Guha, 1975
	2·04 II +43·92 I	..	Nil	Present author
L. floriburda Jack.	..	48	..	Nanda, 1962
L. speciosa Pers.	..	50	..	Bowden, 1945



Figs. 1–5. Figs. 1–4. Metaphase I 24 II *L. flosreginae* Retz., *L. parviflora* Roxb., *L. thorelli* Gagnep. and *L. indica* Linn. respectively. Fig. 5. Metaphase I 2 II + 44 I *L.* × *lancesteri* Hort. (All × 1500).

Unlike that in the species, the meiotic behaviour of the hybrid *L.* × *lancesteri* Hort. is abnormal as at metaphase I, an average of 2·64 II + 43·92 I was observed. (Fig. 5; Table I). Anaphase I is highly irregular with a number of univalents lagging or showing precocious disjunction. The presence of an average of only 2·64 II and the high frequency of univalents (43·92) indicates that the two genomes involved in the origin of the hybrid are cytogenetically well differentiated. The hybrid has been reported to have envolved through interspecific hybridization between *L. matthewsii* Matthews. (Syn. *L. eavesii* Eaves.) and *L. indica* var. *candida* Linn. (Anonymous[1], 1959).

There is complete pollen and seed sterility and the cultivar is propagated through stem layerings. The present studies on this hybrid confirm the observations of Guha[5] (1975). However, intercellular numerical variations in chromosomes as reported by her in somatic tissue and the pollen mother cells was not seen in the material studied here.

Tjio[7] (1948) reported the chromosome number in *L. flosreginae* Retz. as 44, however, in the present taxon of the species investigated here, it was found to be 46, which is in confirmation with the findings of Nanda[6] (1962). The chromosome number in *L. indica* Linn. was found to be 48 which is at variance with the observations of Bowden[3] (1945) who reported $2n = 50$. Due to the non-availability of the material of *L. floribunda* Jack. and *L. speciosa* Pers. it has not been possible to confirm the chromosome number reports of Nanda[6], (1962); and Bowden[3] (1945) for these species.

From the foregoing observations therefore it appears that the genus is based on $X = 24$ which itself may be of polyploid origin.

The author is thankful to Dr. T. N. Kho Director, for encouragement and facilities, Dr. Pal for help and to Shri T. K. Sharma for the trations.

Cytogenetics Laboratory,
National Botanic Gardens,
Lucknow,
June 6, 1977.
REHANA A

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1. Anonymous, *Lagerstroemia*, Bull. No. 28. Nati Botanic Gardens, Lucknow, 1959.
2. Bolkhovskikh, Z., Grif, V., Matvejeva, T. Zakharyeva, O., *Chromosome Numbers Flowering Plants*, Academy of Sciences of USSR, 1969.
3. Bowden, W. M., *Am. J. Bot.*, 1945, **32**, 81.
4. Darlington, C. P., and Wylie, A. P., *Chromo Atlas of Flowering Plants*, George Allen Unwin Ltd., London, 1955.
5. Guha, S., *Proc. Indian Sci. Cong.* (Abstr 1975.
6. Nanda, P. C., *J. Indian Bot. Soc.*, 1962, 41,
7. Tjio, J. H., *Hereditas*, 1948, **34**, 135.

REVIEWS AND NOTICES OF BOOKS

Exploration Geophysics—An Outline. By D. V. L. S. Bhimasankaram, (Assn. of Expln. Geophys; Osmania Univ. Press, Hyderabad), 1977. pp. 111. Price Rs. 50.

Amongst the very few books which have been published furnishing introductory level discourses on Exploration Geophysics (or Geophysical Prospecting), the book under review may be said to be distinguished for its brevity and style. The author has made an attempt to present within the compass of only 109 pages the entire gamut of exploration Geophysics highlighting the salient features of the subject. The book has five chapters—the first (23 pp.) presents a brief account of the general principles and classification of the several geophysical methods. Next, the geological applications in the petroleum and mining fields, and ground water and engineering investigations are covered (32 pp), citing also a couple of examples for each case. The chapter on field operations (14 pp.) points out the general aspects such as the ground, aerial and marine surveys, and also the lay-out of grid and route surveys, profiling and sounding techniques. The spacing of observation points is also discussed to some extent. The remaining two chapters are devoted to Geophysical Anomalies (15 pp.) and Interpretation of data (19 pp). In these two chapters the topics dealt are corrections applied to mea quantities, detectability, factors, the noise pro qualitative and quantitative methods of interp tion, ambiguities and integrated geophysical in gations and finally the planning of surveys and or sation of field parties.

On the whole, the coverage of topics though ap ing somewhat sketchy, could be adequate espe for introducing the subject to the first year stu of geophysics course, who would in any case, learn the subject extensively and in depth.

The illustrations, numbering a total of 70, a good and the lengthy explanation to each p in small types, separated from the text, is an a tage to the reader.

There are hardly any errors of printing or oth which the reviewer came across in reading this One minor omission may however be mentioned re ing the schematic representation of the branch geophysics (fig. 1–1, p. 2): Volcanology and Tec physics which have also been long recognis branches of geophysics, do not find a place in Schematic diagram.

The printing and get up of the Book is very and attractive.

M. B. R

Annual Review of Entomology, Volume 22, Edited by Thomas E. Mittler, Carroll N. Smith and Vincent H. Resh. (Annual Reviews, Inc., Palo Alto, California 96306 U.S.A.), 1977. Pp. 576. Price: USA $ 12.00, Foreign $ 15·50.

The Annual Review of Entomology—Volume 22, 1977 contains 24 valuable articles well written by eminent scientists in their respective fields of specialisation. The articles cover various aspects of economic entomology, biological control/parasitology, taxonomical, toxicological and general aspects of the subject-matter. Apart from the author and subject indexes, cumulative index of authors along with their chapter titles from volumes 13–22 in a well arranged manner are a welcome feature of this volume.

The topics that have been reviewed in this volume are :

(1) Biological control of Forest Insects, (2) Contemporary views of the Inter-relationships between Fleas and the Pathogens of Human and Animal Diseases, (3) Biology and Bionomics of Blood sucking Creatopogomids, (4) Dynamic aspects of Insect-Insecticide Inter-reactions, (5) Dynamics of Larch Bud Moth Populations, (6) Techniques for the Evaluation of Insect Repellents, (7) Hormonal Regulation of Larvae Diapause, (8) Extension Entomology: A Critique, (9) Biosystematics of Trichogramma and Trichogrammato-idea species, (10) Pesticides and Pollinators, (11) Sorghum Entomology, (12) The Peritrophic Membranes of Insects, (13) Model Ecosystem Approach to Insecticide Degradation: A Critique, (14) Evolutionary Genetics of the Hymenoptera, (15) Quality Control in Mass Rearing, (16) Factors Affecting Feeding by Blood sucking Insects, (17) Arthropod Problems in Recreation Areas, (18) Grape Insects, (19) Responses of Lepidoptera to Synthetic Sex Pheromone Chemicals and Their Analogues, (20) Cytogenetics of Mites and Ticks, (21) Influence of Population Density of Size, Fecundity and Developmental Rate of Insects in Culture, (22) Cotton Insect Pest Management, (23) Degradation of Organophosphorous and Carbamate Insecticides in the Soil and by Soil Micro-organisms and (24) Efficacy of Inundative Releases.

I have gone through all the articles and each review is an important standard contribution in its respective branch of Entomology. Special emphasis of the readers may be drawn to the Chapters 1, 2, 4, 6, 8, 9, 10, 11, 13, 18, 22, 23 and 24 which are of applied nature in Economic Entomology. The rich experience of Ray F. Smith who has relinquished his leadership as Senior Editor after 21 years of unceasing efforts has contributed its due part in maintaining such a high standard of the review.

This valuable volume is rather out of reach for individual purchase but must be purchased by all the libraries of Research Institutes and Colleges for the benefit of students and research workers.

V. G. PRASAD.

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ORIGIN AND AGE OF TEKTITES

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ABSTRACT

Tektites from Australia, Thailand and Czechoslovakia have been dated by fission track method. Australites and Thailandites seem to be genetically related but Moldavites belong to a different age group. Various hypotheses on the origin of tektites are discussed. Moon appears to be most plausible extraterrestrial source of these exotic glass pieces.

INTRODUCTION

TEKTITES (from Greek word tektos—molten) are usually small, glassy objects, jet black in colour, and found in certain restricted areas of the world called 'strewn fields'. These are four such fields generally recognised. They consist of: (1) Moldavites, found in the valley of the river Moldau, in Western Czechoslovakia. (2) Australites, discovered in Australia and Indo-China, collectively called the "Australasian strewn field". (3) Ivory coast tektites in western Africa, and still very rare. (4) North American tektites called bediasites from Texas and Georgia.

What makes these chips of 'glass' so interesting from a scientific point of view? First, of course, is their distribution in only restricted fields and second, their peculiar composition. They have distinct physical and chemical characteristics which set them apart from both volcanic glasses (obsidians) and glasses produced on the earth by the impact of meteorites (impactites). Most tektites have shapes which strongly suggest modelling by aerodynamic forces when they traversed the Earth's atmosphere at great speeds, getting their anterior surface ablated in the process. Australites studied by the author show these special characteristics and are generally button-shaped or lensoid in shape.

ORIGIN OF TEKTITES

The most controversial aspects of tektites are the theories of their origin. Two schools of thought have existed for a long time—the terrestrial and the extra-terrestrial. Any theory that seeks to establish their origin must account for the following crucial features:

(1) exsistence of restricted strewn fields; (2) aerodynamic shaping; (3) similarity of tektites from one strewn field to another; (4) total absence of water content, and (5) dissimilarity of tektites with local rocks.

The theories most worthy of consideration are those proposing the formation of tektites by the impact of meteorites or comets, either on the surface of earth or the moon. L. J. Spencer[1] was the first to propose that tektites resulted from colossal meteorite impacts on the earth, the splashed material coming down in a fine spray. There are several objections to this hypothesis: (1) the strewn fields are too large for tektites to achieve the requisite high velocities without complete destruction; (2) if tektites have resulted from fused local rocks, they should reflect their composition whereas they often do not; (3) there should be an impact crater corresponding to each strewn field.

Terrestrialists have given two answers to these objections: The first is that of A. J. Cohen[2] of the University of Pittsburgh who has argued that Ries crater in south of Germany and the Lake Bosumtwi crater in Ghana are responsible respectively for the Moldavites and the Ivory Coast tektites. H. Urey[3] has put forward the ingenious hypothesis of cometry impacts. Comets being so nebulous, consisting largely of heated gases and vapours, no concrete craters need be made.

The extraterrestrialists have advanced the following arguments in support of their hypothesis: (*i*) Verbeek[4] had first proposed the moon as the source of tektites, he imagined them as ejecta from the lunar volcanoes. (*ii*) H. H. Nininger[5] first put forward meteoritic impacts on the moon as the causative agency. (*iii*) Recently D. Chapman[6] has pinpointed not only the crater on the moon (*viz.*, Tycho) which is responsible for the Australasian tektites but also the crater ray (Rosse ray) along which the ejected lunar material initially travelled.

The mystery of origin of tektites is as yet unsolved. On balance, however, the lunar hypothesis appears to be more plausible, as it explains more observed facts. That the moon is the most plausible of extraterrestrial sources is borne out by the following facts: (*i*) the greater probability with which any material ejected from it would find its way to the earth, (*ii*) absence of cosmic ray induced tracks in tektites, (*iii*) aerodynamic shaping and ablation on entry into earth's atmosphere, and the most important of all, (*iv*) resemblance in chemical composition of tektites with lunar rocks.

AGE OF TEKTITES

A number of methods have been used to determine the age of tektites, the most reliable being perhaps the K–Ar and the fission track analysis methods. These two methods have usually yielded age broadly similar to each other.

Since its introduction by Price and Walker[7] the fission track method has proved to be suitable for glasses. Applied to tektites, the method yielded ages quite similar to the K–Ar ages[8-10].

EXPERIMENTAL PROCEDURE

Tektite samples collected from western Australia, Thailand and Bohemia (Czechoslovakia) were purchased from Ward's Natural Science Inc., New York, for fission track analysis. The samples which were jet black in colour became transparent and bottle green on polishing. The highly polished sections of the tektite glasses were etched in 48% hydrofluoric acid at room temperature for 2–5 minutes, depending on the SiO_2-content of the samples. The etched samples were scanned under Carl Zeiss Microscope, for fossil fission tracks due to spontaneous fission of U^{238}. It was found that fossil track density is minimum in Australian tektites and maximum in Bohemian samples. The fossil tracks are circular or elliptical etch pits clearly distinguishable from numerous surface dislocations.

The samples were heated in a muffle furnace at 500° C for one hour to anneal out all the fossil tracks. After annealing, the samples were irradiated in CIRUS Reactor of BARC, Trombay, Bombay, with a thermal neutron dose of 1 (nvt). The irradiated samples were etched as fossil tracks and the number of induced fiss tracks due to induced fission of U^{235} was coun using a 5 × 5 graticule in the microscope eyepi

The fission track ages of tektites were calcula by using the following age formula[11]:

$$T = 6\cdot57 \times 10^{9} \log_e \Big(1 + 9\cdot25 \times 10^{-18} \times \frac{\rho_s}{\rho_i} \times$$

The f.t. ages are summarized in Table I.

TABLE I

Fission track ages of tektites

Total thermal neutron dose $\psi = 1\cdot33 \times 10^{15}$

Tektite sample	No. of Tracks Fossil ρ_s	Induced ρ_i	F.T. age (m.y.)	K-Ar age (m.y.)
Australites	104	12800	0·65±0·06	
	114	13888	0·66±0·06	0·72±0·04
	152	19392	0·63±0·05	
		Mean	0·65±0·06	
Thailandites	332	36928	0·72±0·04	
	113	12928	0·70±0·06	0·72±0·10
	226	28160	0·64±0·04	
		Mean	0·69±0·05	
Maldavites	4624	25664	14·40±0.20	
	4880	28416	13·67±0·21	14·60±0·70
	6400	32640	15·67±0·20	
		Mean	14·58±0·20	

DISCUSSION OF RESULTS

(1) The f.t. ages of Australian tektites Thailand tektites show very good agreement firming the fact that they are genetically rela Therefore, Australasian strewn field is very extending from Western Australia to Indo-Chin

(2) Moldavites (Tektites of Bohemia) belon a different generation with mean f.t. age 14·58 ± 0·20 m.y.

(3) In general, our f.t. age results agree those determined by other authors[8-10].

(4) The mean etch pit diameter of core sam is more than that of flange samples due to int thermal annealing at the flanges during ablation

(5) No evidence is found in favour of a sec tektite fall in Australia as reported by Fleis *et al.*[12].

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1. Spencer, L. J., *Mineral. Mag.*, 1933, 23, 387.
2. Cohen, A. J., *Tektites*, Ed.; J. A. O'Keefe, Univ. of Chicago Press, Chicago, Ill., 1963, p. 189.
3. Urey, H. C., *Nature*, 1963, 197, 228.
4. Verbeek, D. M., *Koninkl, Ned. Akad. Wetenschap. Proc. Ser.*, 1897, B 5, 421.
5. Nininger, H. H., *Sky Telescope*, 1943, 2 (4), 12.
6. Chapman, D. R., *Abstracts 3rd International Tektite Symp.*, Corning, N.Y., 1969, p. 8.
7. Price, P. B. and Walker, R. M., *J. Geophys. Res.*, 1963, 68, 4847.
8. Fleischer, R. L. and Price, P. B., *Geochim. Cosmochim. Acta*, 1964, 28, 755.
9. — and —, *Ibid.*, 1965, 29, 161.
10. Gentner, W., Kleinman, B. and Wagner, G. A., *Earth Planet. Sci. Lett.*, 1967, 2, 83.
11. Virk, H. S. and Koul, S. L., *Curr. Sci.*, 1975, 44, 211.
12. Fleischer, R. L., Price, P. B. and Woods, R. T., *Earth Planet. Sci. Lett.*, 1969, 7, 51.

PERMEABILITY THROUGH AN ANIMAL MEMBRANE

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ABSTRACT

Transport of various non-electrolytes, namely, water, acetamide, urea, glucose and sucrose has been studied through a urinary bladder of goat. it has been found that the flow through the membrane is neither diffusive nor viscous but flow through certain number of capillaries of membrane is diffusive and viscous through the rest.

INTRODUCTION

TRANSPORT across natural membranes is a complex phenomenon[1,2]. Since the pore size in such membranes is comparable to the molecular size and channels have undefined geometry and complex surface characteristics, experimental studies are difficult. Earlier studies on transport processes, in both artificial[3] membranes (analogous to the natural membranes[4,5]) and in natural membranes[6], have revealed some useful information. From these studies on permeability of membranes, it can be assessed[7]—(*i*) whether the movement occurs through the bulk of the membrane or through specific limited region of membrane, (*ii*) whether it is brought about by the action of the specific membrane components which are of use in explaining structural relationship of the membrane with permeating species.

With these objectives, an experiment on membrane transport is described in the present communication, using the urinary bladder of a goat in presence of water, urea, acetamide, glucose and sucrose.

EXPERIMENTAL

The urinary bladder of goat was equilibrated with urea solution and fixed in rubber gaskets[8]. The flow volume was measured by noting the rate of advancement of liquid meniscus in a capillary of radius 0·024 cm. The solutions were preheated to the temperature (25° ± 0·01° C) of the experimental cell. The diffusion coefficients were calculated from the viscosity data of solutions as suggested earlier[9].

RESULTS AND DISCUSSION

Transport equation for the flow of matter in presence of pressure difference alone through a membrane reduces to[10]

$$J_v = L \triangle P \qquad (1)$$

where J_v is the volume flow of matter, $\triangle P$ is the pressure difference across the membrane and L is the permeability coefficient.

For viscous flow the equation can be written as

$$J_v = \frac{n \pi r^4}{8 \eta l} \triangle P \qquad (2)$$

where n, r, η and l represent number of capillaries, radius of the capillaries in the membrane, viscosity of solution and length of the capillaries in the membrane respectively. Comparison of Eqs. (1) and (2) gives the following relationship.

$$L = \frac{n \pi r^4}{8 \eta l} \qquad (3)$$

The values of L have been calculated from the slope of a plot between J_v and $\triangle P$ and are recorded in Table I. Although it is expected that L should decrease by increasing the molecular weight of the solutes, yet this has not been found to be valid in the present case. It is obvious from Eq. (3) that

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L should decrease with an increase in η. This appears to be the cause for the unexpected variation of L with molecular weight of the solutes.

TABLE I

The values of various constants for different permeating species at 25°C. Concentration of all the permeating solutions was 0·1 M.

Permeating species	$L \times 10^8$ (Cm^5 dyn.$^{-1}$ sec.$^{-1}$)	$K_v \times 10^8$ (Cm^5 dyn.$^{-1}$ sec.$^{-1}$) poise	$K_d \times 10^8$ (g. dyn.$^{-1}$)
Water	0·772	0·685	0·323
Acetamide	0·666	0·752	0.622
Urea	0·650	0·598	0·424
Glucose	0·731	0·710	0·835
Sucrose	0·605	0·635	0·731

In the case of diffusive flow the equation representing the transport of matter can be written as[11]

$$J_v = \frac{D\epsilon v}{RTl}\,\Delta P \qquad (4)$$

where D is the diffusion coefficient and ϵ is the fractional void volume. Comparing Eqs. (1) and (4) we get

$$L = \frac{D\epsilon v}{RTl} \qquad (5)$$

Separating the variables D, η and v from Eqs. (3) and (5) we can rewrite Eqs. as follows

$$L \times \eta = \frac{n\pi r^4}{8l} = K_v \,(\text{say}) \qquad (6)$$

and

$$\frac{L}{Dv} = \frac{\epsilon}{RTl} = K_2 \,(\text{say}) \qquad (7)$$

If the flow through certain number of pores, n' is viscous and diffusive through the rest, the permeability coefficient can be written as[12,13].

$$\frac{L}{Dv} = \frac{n'\pi r^4}{8\eta l Dv} + \frac{\epsilon}{RTl} \qquad (8)$$

The role of pore size in the membranes becomes quite important when a permeating species enters the membrane. The three distinct cases are listed below :–

(*i*) Pore size ≫ Mean free path of permeating molecules	Poiseulle's flow
(*ii*) Pore size ≪ Mean free path of permeating molecules	Knudsen flow
(*iii*) Pore size ≈ Mean free path of permeating molecules	Slip flow

A suitable explanation of the type of flow be given in terms of Eqs. (6) and (7) in variables are η, D and v. If the values of K_d or K_v are constant, the flow will be diffusive or viscous[14]. It is obvious from T that the values of neither K_d nor K constant. This implies that the flow is n diffusive nor viscous.

The data have been further utilized to ex whether the flow through n' pores is viscou diffusive through the rest. A plot between L *vs.* $L/\eta D$ is a straight line with an intercep L/Dv-axis in accordance with Eq. (8). It be concluded that the flow through cerlain nu of pores is diffusive and viscous through the In other words, if is the case of slip flow.

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1. Lakshminarayaniah, N., *Transport Phen in Membranes,* Academic Press, New 1969.
2. Rastogi, R. P., *J. Sci. and Ind. Res.,* 35, 64.
3. Garby, L., *Nature,* 1955, 173, 444.
4. Srivastava, R. C. and Jain, A. K., *Indi Chem.,* 1975, 13, 1306.
5. Jain, A. K., Upadhyay, S. K. and Sriva R. C., *J. Non-Equilibrium Thermodyn* Accepted.
6. Anderson, B. and Ussing, H. H., *Acta Ph Scand.,* 1957, 39, 228.
7. Stein, W. D., *Movement of Moltcules A Cell Membranes,* Academic Press, York, 1967.
8. Srivastava, R. C. and Jain, A. K., *J. Po Sci. Polymer Phys.,* Ed., 1975, 13, 16
9. Li, J. C. M. and Chang, P., *J. Chem. P* 1955, 23, 578.
10. Katchalsky, A. and Curran, P. F., *Equilibrium Thermodynamics in Bio--Ph* Harvard University Press, Cambridge,
11. Tickner, L. B., *J. Phys. Chem.,* 1958, 1483.
12. Meson, E. A., *J. Chem. Phys.,* 1967, 3199.
13. Present, R. D., *Kinetic Theory of G* Mcgraw-Hill, New York, 1958.
14. Rastogi, R. P., Singh, K. and Shukla, P. *Indian J. Chem.,* 1971, 9, 1372.

THE SYNTHESIS AND PROPERTIES OF 2-*m*-TOLYLAMINO-4-*m*-TOLYIMINO-2-PENTENE COMPLEXES OF THE TRIPOSITIVE LANTHANIDE IONS

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ABSTRACT

The reaction of 2-*m*-tolylamino-4-*m*-tolyimino-2-pentene (TTP) with a lanthanide (III) nitrates in anhydrous acetonitrile yields solid compounds of the types $[Ln(TTP)_4](NO_3)_3$; $[Ln(TTP)_4NO_3](NO_3)_2$; and $[Ln(TTP)_3(NO_3)_2](NO_3)$; where Ln^{3++} = La, Pr, Nd, Sm, Er, and Yb and TTP = 2-*m*-tolylamino-4-*m*-tolyimino-2-pentene. Nitrate ion coordination in several of the complexes is established by infrared data. The decrease in metal ion radius across the lanthanide series does not affect the number of ligand molecules coordinated to the Ln^{3+} ion, but the coordination by nitrate is affected. The result is a variation of total coordination number of the cation.

INTRODUCTION

LANTHANIDE (III) nitrate chelates, with 2-phenylamino-4-phenylimino-2-pentene, 2-benzylamino-4-benzylimino-2-ene, etc., were recently synthesised and characterised[1-2]. In this communication, the isolation and characterisation of lanthanide nitrate chelates with 2-*m*-tolylamino-4-*m*-tolylimino-2-pentene (TTP) are described.

EXPERIMENTAL SECTION

Reagents

The anhydrous lanthanide (III) nitrates were prepared from lanthanide oxides[3]. Reagent grade acetonitrile was dried by distillation with phosphorus pentoxide. TTP was prepared by the procedure reported elsewhere[4].

Synthesis of the complexes.—A solution of TTP in acetonitrile (12 mM for tetrakis, and 6 mM for tris chelates) was added slowly to a well stirred lanthanide nitrate solution (2 mM) in acetonitrile employing a dry box. The precipitate thus formed was refluxed for 8 hr for completion of the reaction.

Analysis.—Carbon, hydrogen, and nitrogen were estimated by micro methods. Metal estimations were carried out by standard procedures[5] A complete list of lanthanide chelates and their analytical data are given in Table I. The infrared spectra were recorded on a Perkin–Elmer infracord spectrophotometer model 521 in KBr pellets.

RESULTS AND DISCUSSION

Infrared Spectra.—The infrared spectra of EEE chelates of the lanthanide (III) nitrate in the regions of nitrate absorption are given in Table II. Assignments of the nitrate ion absorption are

TABLE I

Compounds prepared and the analytical data

Compounds prepared*	Colour	% Metal		% C		% H		% N	
		Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
$[La(TTP)_4NO_3](NO_3)_2$	White	9·64	9·85	63·28	63·36	6·38	6·30	10·69	10·56
$[Pr(TTP)_4NO_3](NO_3)_2$	Green	9·77	9·99	63·20	63·10	6·37	6·32	10·68	10·60
$[Nd(TTP)_4NO_3](NO_3)_2$	Violet	9·97	10·15	63·05	62·88	6·36	6·46	10·64	10·58
$[Sm(TTP)_4NO_3](NO_3)_2$	White	10·33	10·48	62·81	62·86	6·33	6·39	10·60	10·40
$[Er(TTP)_4](NO_3)_3$	Pink	11·37	11·48	62·09	62·25	6·25	6·29	10·48	10·46
$[Yb(TTP)_4](NO_3)_3$	White	11·73	11·99	61·84	61·74	6·23	6·32	10·44	10·30
$[La(TTP)_4(NO_3)_2](NO_3)$	White	11·96	12·16	58·87	58·78	5·93	6·16	10·84	10·78
$[Pr(TTP)_3(NO_3)_2](NO_3)$	Green	12·12	13·08	58·76	58·80	5·92	6·04	10·82	10·83
$[Nd(TTP)_3(NO_3)_2](NO_3)$	Violet	12·34	12·45	58·62	58·60	5·91	5·89	10·80	10·70
$[Sm(TTP)_3(NO_3)_2](NO_3)$	White	12·78	12·94	58·32	58·40	5·88	5·80	10·74	10·70
$[Er(TTP)_3(NO_3)_2](NO_3)$	Pink	14·04	15·01	57·49	57·32	5·79	5·80	10·57	10·60
$[Yb(TTP)_3(NO_3)_2](NO_3)$	White	14·46	15·10	57·22	57·18	5·77	5·76	10·54	10·46

* Designation of coordination sphere dictated by i.r. spectral data.

TABLE II

*I.R. Data for '3' representative complexes indicating the nature of the nitrate groups (in cm^{-1})**

Complex	ν_2	ν_3	ν_4	$\nu_1 + \nu_4$	$\nu_1 = \nu_3$	$2\nu_1$	Nature of t Nitrate gro
$[La(TTP)_4NO_3]$	820 (m)	1360 (s)	700 (w)	1750 (vw)	2382 (vw)	2055 (vw)	Ionic
$(NO_3)_2$	796 (m)	1350 (s)	708 (w)	1710 (vw)	2270 (vw)		Monodenta
		1490 (s)	720 (w)	1725 (vw)	2415 (vw)	2050 (vw)	
$[La(TTP)_4](NO_3)_3$	832 (m)	1355 (s)	706 (w)	1750 (vw)	2410 (vw)	2045 (vw)	Ionid
$[La(TTP)_3(NO_3)_2]$	828 (m)	1365 (s)	704 (w)	1748 (vw)	2375 (vw)	2060	Ionic
$(NO_3)_2$	809 (m)	1290 (s)	690 (vw)	1723 (vw)	2290 (vw)	2070	Bidentate
		1495 (s)	746 (m)	1758 (vw)	2545 (vw)		

* Assignments based on ref. no. (7).

based upon two possible symmetry types, D_{3h} (free nitrate) and C_2, coordinated nitrate[6-8].

All the tris chelates show absorptions characteristic of both the ionic and bidentate nitrate groups and hence the complexes have been formulated as $[Ln(TTP)_3(NO_3)_2](NO_3)$ on the basis of steric grounds. The infrared spectra of these chelates show a greater weakening of ν_3 greater splitting of ν_3 and ν_4 and large shift of ν_2 to lower frequency.

The lighter tetrakis chelates La^{3+} to Sm^3 have absorption characteristic of both ionic and monodentate nitrate groups and hence the chelates have been formulated as$[Ln(L)_4NO_3](NO_3)_2$ on the basis of steric grounds. The infrared spectra of the nitrate ions are similar for the chelates, the main differences from bidentate chelates being the considerable weakening of ν_3, splitting of ν_3 and ν_4 and a small shift of ν_2 to lower frequency.

The complexes $Ln(TT)_4](NO_3)_3$, (Ln = Er and Yb) with eight amine donor groups show bands corresponding only to ionic nitrate groups. It is apparent that as the radius of the lanthanide ion decreased, the single coordinated nitrate group in the tetrakis chelates is forced out from the coordination sphere due to steric crowding of the amine ligand molecules.

The tetrakis chelates of heavier lanthanides show strong absorption at 3240 cm^{-1}, which is shifted by 50 cm^{-1} towards the higher frequency when a nitrate group is introduced into the coordination sphere (La^3 to Sm^{3+}). A slight splitting of this band is also observed. The effect of coordination is clearly demonstrated for tris chelates (bands observed at ~ 3298 cm^{-1}) in which three well resolved bands are observed. In each case however, the stretching frequencies of coordinated groups is less than the free ligand value (3328 cm This may be attributed to the combined effect coordination to a positive metal ion[9] and hydro bonding between the anion present and the proton[10]. The large number of amino ba observed upon nitrate coordination may attributed to non-equivalent sets of amino gro The increase in frequency of one set may re from repulsions between the nitrogen end of NH dipole and the coordinated nitrate ion poorer hydrogen bonding of the amino prot with the coordinated nitrate ion.

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1. Mohan, M., *Ann. Chim.*, 1975, 65, 533.
2. —, *Indian J. Chem.*, 1977, 14 A, 247.
3. Moeller, T., Aftandilian, V. D. and Cull G. W., *Inorg. Synth.*, 1957, 5, 37.
4. Parks, J. E. and Holm, R. H., *Inorg. Che* 1968, 7, 1408.
5. Srivastava, A. K., Rana, V. B., Mohan, Swami, M. P. and Jain, P. C., *J. In Nucl. Chem.*, 1975, 37, 723.
6. Ferraro, J. R., *J. Molec. Spectrosc.*, 1 4, 99.
7. Curtis, N. F. and Curtis, Y. M., *Inorg. Che* 1965, 4, 804.
8. Lever, A. B. P., Mantovani, E. and Ra swamy, B. S., *Cand. J. Chem.*, 1971, 1957.
9. Svatos, G. E., Curran, C. and Quaglia J. V., *J. Am. Chem. Soc.*, 1957, 6159.
10. Bellamy, L. J., *The Infrared Spectra of Co plex Molecules*, Wiley, New York, 19 p. 379.

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CHROMOSOME NUMBER OF SOME GARDEN ROSES

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ABSTRACT

Realising the necessity to have some quick and less cumbersome method for obtaining mitotic chromosomes, a technique was standardised to obtain mitotic preparations from leaf tips. This was found to be extremely useful as the mitotic chromosomes can then be studied all the year round, for the leaves are available throughout the year in roses. Based on this technique chromosome numbers of some varieties have been listed and for those the number of which have been already reported from mitotic preparations, it has been confirmed.

INTRODUCTION

INFORMATION on the chromosome number of garden roses is lacking in most cases. Apart from reports by Flory[1] (1950), Wylie[2] (1954), Rowley[3] (1961), Shahare and Shastry[4] (1963) and few others on the chromosome number of some garden roses, most of the cultivars have not been analysed for this purpose. Since the chromosome number and the ploidy level of the cultivar is one of the basic needs to plan an improvement programme, it was considered worthwhile to examine chromosome numbers of some of the common cultivars.

MATERIALS AND METHODS

Twenty varieties of garden roses available at Rose Collection of I.A.R.I., New Delhi, and Experimental Plot of Dr. B. P. Pal situated at Indian Agricultural Research Institute, New Delhi, were studied. Names of the varieties and the horticultural class to which they belong are presented in Table I, Columns 2 and 3.

The following schedule was successfully developed for obtaining good mitotic preparations from young leaf tips.

1. Collect the youngest leaves which can be manipulated easily. Separate them out from the cluster and wash in water.
2. Immerse these in glass phial containing saturated solution of para-dichloro-benzene for pretreatment. This was prepared by dissolving 7·5 gm of para-dichloro-benzene in 100 ml of water, heating it slightly to dissolve and filtering after it cools down. Leave the leaves for two hours at room temperature. Wash thoroughly in water.
3. Fix the leaves in acetic alcohol (1 : 3) for three hours. Change the fixative with 1 : 1 acetic alcohol. Change the fixative once or twice till the chlorophyll is completely removed. Leave overnight.
4. Heat the leaves in a mixture of 2% aceto-orcein and normal HCl (9 : 1) over a flame, leave for 3–4 hours ; they can be left safely overnight also.
5. Keep the leaf on a clean dry slide, dissect out the tip portion removing all the midrib portion to get good spreading, and squash in 1% acetoorcein tapping at the cover slip. Very small amount of tissue should be taken to get good spreading.

RESULTS AND DISCUSSION

Table I listed the chromosome number of twenty cultivars studied from mitotic preparations.

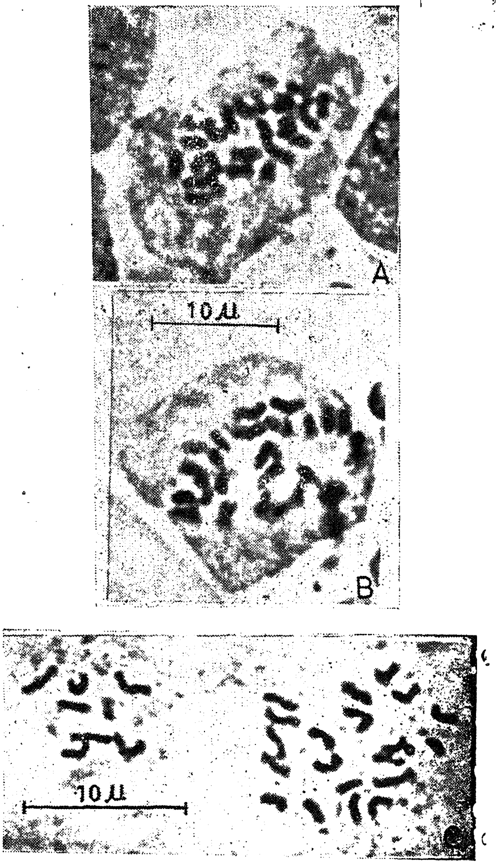
TABLE I

Showing chromosome number of garden roses as determined from mitotic preparations

Sl. No.	Name of the cultivar	Class	Chromosome number (2 *n*)
1.	Charles Mallerin	HT	28
2.	Chitchor	Fl	21
3.	Crimsom Glory	HT	28
4.	Crown Princess Victoria	HT	21
5.	Docteur Valois	HT	28
6.	Etoile de Hollande	HT	28
7.	Flaming Sunset	HT	28
8.	Ganga	HT	28
9.	Goudvlinder	HT	28
10.	Happiness	HT	28
11.	Kiss of fire	HT	28
12.	Lady Frost	HT	28
13.	Michéle Meilland	HT	28
14.	Mirandy	HT	21
15.	Montezuma	Gr	28
16.	Queen Elizabeth	Gr	28
17.	Soraya	HT	28
18.	Spartan	Fl	28
19.	Super Star	HT	28
20.	Una Wallace	HT	28

Abbreviations :
HT—Hybrid tea ; Fl—Floribunda ; Gr—Grandiflora.

Figures A–C show the mitotic preparations obtained in this way.



FIGS. A–C Mitotic chromosomes ($2n = 28$) Fig. A. 'Queen Elizabeth'. Fig. B. 'Super Star'. Fig. C. 'Kiss of Fire'.

Earlier workers have studied the chromosome number in roses from an examination of the pollen mother cells. This technique has its own limitations. For example, the floral buds are available only during a restricted period. Also meiosis in the case of many garden roses shows various irregularities which make it difficult at times t determine the number correctly. The small si and the heteromorphic nature of chromosomes ar other factors responsible for difficulties in corre determination of chromosome number. Hence, i was thought necessary to develop a schedule t determine the chromosome number from mito preparations.

The root tips which are widely used for mito studies in many other plant materials are difficu to procure in roses, as the cuttings of most the cultivars do not root easily. Moreover, it a time consuming process. The seeds are al difficult to germinate as they show dormancy a only a small fraction germinates. Moreover, t seeds do not represent the correct ploidy level the parental plant, as they are a result of crossi with various other cultivars. For these reas the above mentioned schedule was developed get good mitotic preparations from young leaf ti which are available almost all through the year.

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1. Flory, W. S. and Virginia, *J. Sci.,* 1 1, 11.
2. Wylie, A. P., *American Rose Annual,* 1 54, 36.
3. Rowley and Gordon, *J. Genet.,* 1960, 253.
4. Shahare, M. L. and Shastry, S. V. *Chromosoma,* 1963, 13, 702.

TECHNOLOGY AND THE SCIENCES

THE Government of India appears to be taking a new look at science. The reconstitution of the National Committee on Science and Technology (NCST) is its latest effort in this direction.

That India has made tremendous progress since independence in science and technology has been recognized by several unbiased observers. The great variety of industrial products which she can manufacture has astonished many advanced countries and the unqualified praise for her ability to produce sophisticated equipment, accorded by the highly industrialized nations, has been genuine as well as unequivocal. India has emerged during this period as a strong exporter of finished goods of consumer interest. The employment of her technical abilities to manufacture a great variety of products has conferred on India the distinction of a potentially strong competitor in world markets—a position which is looked upon with some apprehension by the traditionally industrial nations of the world.

The association of science with technology and industry-biased research is an old and well-established historical fact. Our early planners clearly saw the link between basic science and its applications. In the constitution of a number of laboratories under the CSIR and in the encouragement given to agriculture, medicine and defence, this role of science was recognized. Our technical and industrial development is not a little due to the filip provided in the early days of our independence for not only encouraging science but also emphasizing the urgency of applying it to the industrial advancement of the country. This association clearly has conferred enduring benefits and made India among the top industrialized nations in the world.

NEGLECT OF BASIC SCIENCES

However, in the process, basic sciences have suffered some neglect. Admittedly and for good reason, the emphasis has been on those aspects of the sciences which have a direct bearing on technology. India in its national laboratories has pioneered science relating to textiles, paints, metals, glass, electronic and electrochemical products, drugs, etc. But those branches of science which have no immediate or clear application or possibilities of application have received little prominence or support at the hands of Government. It has been acknowledged that today in India there is a sharp polarization of scientific research in that while industrial research and such scientific research that has possible applications to industry are done in the national laboratories of the CSIR and other agencies, basic research with no immediate possibilities of application is relegated to the universities in the country. This is *per se* not an undesirable situation, and prevails in several advanced countries, where eminent scholars in science work in the universities. Indeed, the large majority of Nobel Prizes in the sciences have gone to university scientists. The universities are recognised as centers of learning where the human mind is encouraged to the acquisition of genuine natural knowledge regardless of its applications. On the other hand, the manipulation of knowledge and the phenomena of the real world for the sake of man is the function of technology and an appreciation of these roles of science and of technology is the foundation on which modernity is built in these advanced countries.

In India, however, this appreciation is lacking and in our enthusiasm to technologically advance the country we have permitted the sciences to suffer neglect. Indeed it is our view that while the lumping together of science and technology has been of considerable benefit to the latter, science itself has suffered. The CSIR does very little basic science outside the scope of industrial application, and the support provided for basic sciences under governmental auspices is negligible. The plea that a 'backward' country like India needs to ensure a decent standard of living for its people, that food, clothing, shelter, health and education are priorities in any scheme of development, is admitted. But India is backward perhaps in the economic field. It is not backward in its science development. How do we reconcile the presence of a very large number of competent scientists in a milieu of poverty and deprived surroundings?

UNIVERSITIES AND SCIENCE

One does not need to be reminded that the structure, organization and administration of the universities in this country, by and large, are hardly conducive for the development of learning, let alone scientific learning. Thoroughly indigent, ridden by parochial, state problems and pulls, governed by unimaginative rules and tenets, too numerous and diffuse, the universities can lay no claim to be temples of learning or venues for the acquisition of natural knowledge. To relegate science and scientific research to such institutions is to stifle all initiative and make science a lifeless drudgery with neither content nor meaning.

SCIENCE AS A CAREER

But more importantly, science in India is not a career and does not provide one. It is tragic that a large number of ‘Science Talent Scholars’ end up, not as dedicated scientists working in the laboratories of the country advancing the frontiers of scientific knowledge, but as officers in the administrative or police service, or in the banking establishments. It is not uncommon to see science students willing and ready to pay huge capitation fees to secure admission to medical, engineering and technological institutions rather than choose a career in science. It is a truism that only those young people who do not secure admissions in technical institutions opt for science. Medicine, Engineering, Agriculture are career-oriented disciplines and one does not wish to blame young people if their priorities do not include science. Science in this country is neither a calling nor a means of livelihood.

We therefore need to take a close look at the sciences and ensure for them a place not only of honour but also a career-providing opportunity. It seems the time has come for Government to acknowledge the place of basic sciences in the growth of our nation and recognize its innovative role in technological advancement as a symbiotic relationship enriching each other but distinct from one another. The concept that support provided for technological or industrial research automatically encourages advancement of scientific knowledge is manifestly incorrect. Technology unrelated to science makes for craftsmanship. Creativity comes from science. If we may draw an analogy from Indian music, technology is virtuosity, mastery of technique, facility of expression and precision of construction. Science is something else, it is creativity, the unexpected flash of genius. Creativity, (Pratibha) and Virtuosity (*Sādhana*) must go together. Both are needed to make a musician. The latter alone—craftsmanship or virtuosity—fails to impress in the absence of innovative creativity. Creativity comes from the mind. Virtuosity is a matter of *Abhyāsa*, an effort of the finger or the larynx. It makes for expert music, not necessarily a pleasing one.

SCIENCE AS CULTURAL COMPONENT

Also, science is an essential component of culture. It is a means of perception of nature, of the world and of the universe. Scientific training sharpens one's perception enabling one to see connections where none was available before, to see the universe more or less as a totality rather than as fragments. To be sure, fragmentation remains but the process of proceeding towards a holistic perception is the flow of science. In this sense, science enriches life, adds to its quality an advances towards efficient, effective and orderl functioning.

It was reported the other day that th reconstituted NCST would direct its attention t rural priorities, to make the rural areas and rur populations in our country adopt scientific method practices and modes of life and to provide ou villages the amenities they have lacked and whic we pledged to provide at the time of attaini independence. These are unexceptionable objectiv and the Chairman and members of the NCST c be expected to fulfil these objectives if resourc are made available. At the same time, it sho be ensured that basic sciences are not lost sig of. Whether by suitably orienting its policies calling on the University Grants Commission the Science Academies, the NCST should qu quickly convince the very large number of work scientists in the country that scientific research its own sake is an endeavour which will not l support from Government.

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LETTERS TO THE EDITOR

ELECTRONIC TRANSITION MOMENT VARIATION IN AlO ($A^2\Sigma^+$–$X^2\Sigma^+$) BAND SYSTEM

PRECISE knowledge of the variation of electronic transition moment (R_e) with internuclear distance (r) is needed for the study of transition probabilities in the band systems of diatomic molecules. The dependence of R_e on r is generally determined by the following expression,

$$I_{v'v''} = KN_{v'}\, \nu^4_{v'v''}\, R_e^2(\bar{r}_{v'v''}) \left| \int \psi_{v'}\, \psi_{v''}\, dr \right|^2$$

or

$$(I_{v'v''}/q_{v'v''}\, \nu^4_{v'v''})^{1/2} = (KN_{v'})^{1/2}\, R_e(\bar{r}_{v'v''}) \qquad (1)$$

where, $I_{v'v''}$ is the intensity of an emission band, $q_{v'v''} = \left| \int \psi_{v'}\, \psi_{v''}\, dr \right|^2$ is the Franck-Condon (FC) factor, and the other symbols carry their usual[1] meaning. Since $N_{v'}$ is a constant for a particular progression, equation (1) can be written as,

$$R_e(\bar{r}_{v'v''}) \propto (I_{v'v''}/q_{v'o''}\, \nu^4_{v'v''})^{1/2}.$$

Using this relation Nicholls and Jarmain[2] have proposed that the dependance of R_e on r can be studied by plotting a graph of $(I_{v'v''}/q_{v'v''}\, \nu^4_{v'v''})^{1/2}$ against $\bar{r}_{v'v''}$. These plots for different v''-progressions are scattered due to the fact that $N_{v'}$ is constant only for a given v''-progression. The rescaling procedure due to Turner and Nicholls[3] is usually adopted to bring these plots on the same scale. However, there is an inherent drawback in this procedure since all the measured intensities of the bands cannot be used. Therefore, the method of regression due to Cunio and Johnson[4] is to be used for the evaluation of R_e as a function of r. In this method all the measured intensities of the bands are used. The present note reports the dependance of R_e on r for the (A–X) transition of AlO molecule using the said method of regression. Recently reported[5] R-K-R-V FC-factors and r-centroids for this transition are used along with the available[6,7] band intensities in different spectral sources and the R_e-r relations are derived. The resulting linear and quadratic expressions are given in Table I. These expressions are valid for the range of r between 1·519 Å to 1·772 Å.

TABLE I

Sl. No.	Spectral Source	$R_e - r$ relations
1.	Low pressure arc	$R_e = k\,(1 - 0{\cdot}482\, r)$ $R_e = k\,(1 - 1{\cdot}759\, r + 0{\cdot}623\, r^2)$
2.	Shock tube	$R_e = k\,(1 - 0{\cdot}391\, r)$ $R_e = k\,(1 - 1{\cdot}298\, r + 0{\cdot}392\, r^2)$
3.	Exploding wire	$R_e = k\,(1\cdot - 0{\cdot}473\, r)$ $R_e = k\,(1 - 1{\cdot}431\, r = 0{\cdot}002\, r^2)$

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1. Herzberg, G., *Spectra of Diatomic Molecules*, (D. Van Nostrand, New York), 1950.
2. Nicholls, R. W. and Jarmain, W. R., *Proc. Phys. Soc.*, 1956, **69A**, 253.
3. Turner, G. R. and Nicholls, R. W., *Can. J. Phys.*, 1954, **32**, 468, 475.
4. Cunio, B. E. and Johnson, R. E. W., *JQSRT*, 1967, 7, 639.
5. Mummigatti, V. M. and Jyoti, B. G., *Curr. Sci.*, 1977, **46** (8), 256.
6. Hebert, G. R. and Tyte, D. C., *Proc. Phys., Soc.*, 1964, **83**, 629.
7. Tyte, D. C. and Hebert, G. R., *Ibid.*, 1964, **84**, 830.

INFRARED SPECTRA OF METHYL BENZOATE AND BENZYL BENZOATE

METHYL BENZOATE and benzyl benzoate are monosubstituted benzenes wherein one hydrogen atom in each of the molecules are replaced by $-COOCH_3$ and $-COOCH_2C_6H_5$ respectively in the position '1' of the benzene ring. The Raman spectra of methyl benzoate and ethyl benzoate were earlier studied by Biswas[1], Matsuno[2], Kohlrausch *et al.*[3]. Since a complete assignment of the observed infrared bands of methyl benzoate (MB) and benzyl benzoate (BB) has not been given by any of the earlier workers[5–8]. A study of the infrared spectra of these molecules in the liquid phase was undertaken to obtain detailed information about the fundamental vibrations in the ground state.

The infrared spectra of MB and BB, contained in a cell, has been recorded in the liquid phase in the region 4000–250 cm^{-1} on a Perkin Elmer double beam Infrared Spectrophotometer. The thickness of the cell was 0·025 mm. The chemicals (by B.D.H., England) were of pure quality and were used as such. The observed bands are analysed in terms of various ground state vibrations.

Molecules of MB and BB are assumed under C_{2v} symmetry where the thirty normal modes of vibrations can be divided into 11, a_1 type; 3, a_2 type; 10, b_1 type and 6, b_2 type modes of vibrations. Along with this there will be some internal vibrations due to substituents. The analysis of bands and the assignment of fundamental frequencies have been proposed on the basis of comparison with those of similar molecules. The assignment of the fundamental modes are given in Table I.

TABLE I

Vibrational frequencies (in cm^{-1}*) and assignment*

Raman frequencies of C_6H_5X $X = COOCH_3$	Infrared frequencies of C_6H_5X		Assignment
	$X = COOCH_3$	$X = COOCH_2C_6H_5$	
218 (5) D	258 (4, s)	263 (4, s)	$b_1 \phi$ (c–X)
	335 (7, vs)	330 (3)	$b_1 \phi$ (c–C)
360 (4) P	359 (5, s)	355 (3)	$a_1 \alpha$ (c–C)
	475 (4)	454 (4, s)	$b_1 \phi$ (c–C)
	519 (2)	595 (5, vs)	$a_1 \alpha$ (c–c)
679* (2) P^1	685 (9, vs)	705 (10, b)	$b_1 \phi$ (c–c)
808 (0)	802 (5, s)	800 (5, s)	b_1 (γ c–H)
826 (6) P	820 (9, vs)	820 (4, s)	b_2 or b_1 γ (c–H)
850 (0)	845 (3)	840 (3, s)	$b_1 \gamma$ (c–H)
942**	932 (5, s)	880 (3, s)	$b_1 \gamma$ (c–H)
969 (1)	965 (8, vs)	908 (7, vs)	b_2 or b_1 γ (c–H)
1003 (10) P	1000 (4, s)	1000 (6, s)	$a_1 \nu$ (c–c)
1028 (4) P	1025 (9, vs)	1021 (10, vs)	$a_1 \beta$ (c–H)
1064 (2)	1070 (9, vs)	1065 (10, vs)	$a_1 \beta$ (c–H)
1111* (3) P^1	1110 (10, b)	1100 (10, b)	$a_1 \beta$ (c–H)
1160 (3) D	1175 (9, s)	1172 (9, vs)	$a_1 \beta$ (c–H)
1277 (8) P	1280 (10, b)	1260 (10, b)	$a_1 \beta$ (c–H)
1311 (3) P	1313 (10, s)	1310 (10, vs)	$a_1 \nu$ (c–c)
1452* (2) D^1	1450 (10, s)	1450 (10, vs)	$a_1 \nu$ (c–c)
1495 (2)	1490 (7, vs)	1495 (8, vs)	$a_1 \nu$ (c–c)
1591 (1)	1580 (7, s)	1582 (7, vs)	$a_1 \nu$ (c–c)
1603 (10) D	1600 (9, vs)	1600 (8, vs)	$a_1 \nu$ (c–c)
	3000 (6, s)	3005 (7, vs)	$a_1 \nu$ (c–H)
	3033 (6, 5)	3030 (7, vs)	$a_1 \nu$ (c–H)
3073 (5 b) P	3063 (6, s)	3064 (7, s)	$a_1 \nu$ (c–H)
	3090 (6)	3090 (5, s)	$a_1 \nu$ (c–H)

Note: Values in parenthesis refer to the relative intensities. ν = stretching, β = in-plane bending, γ = out-of-plane bending, α = in-plane deformation, ϕ = out-of-plane deformation, s = sharp, vs = very sharp, b = broad, * = polarisation data taken from Biswas, ** = frequency taken from Biswas.

C–C Ring Deformation Frequencies: Amongst the C = C stretching, frequencies 1000 cm^{-1} in both the compounds are identified as ring breathing frequency. Along with this, the frequencies 1313, 1450, 1490, 1580, 1600 cm^{-1} and 1310, 1450, 1495, 1582, 1600 cm^{-1} are assigned as the other carbon stretching frequencies for MB and BB, and correspond to 1585 (e_{2g}), 1485 (e_{1u}) and 1310 (b_{2u}) modes of benzene.

C–H Stretching Frequencies: Sharp and very sharp infrared bands appearing at 3000, 3033, 3063, 3090 cm^{-1} and 3005, 3030, 3064, 3090 cm^{-1} have been considered due to C–H stretching for MB and MB respectively.

C–H Bending Vibrations: There are four very sharp, sharp and broad bands for both the compounds at 1025, 1070, 1110, 1175 cm^{-1} in MB and at 1021, 1065, 1100, 1172 cm^{-1} in BB which may correspond to C–H in-plane bending vibrations. The bands observed at 802, 820, 845, 932, 965 cm^{-1} and 800, 820, 840, 880, 908 cm^{-1} may correspond to the C–H out-of-plane bending vibrations for the respective compounds.

C–C Bending Vibrations: The infrared bands at 335, 475, 685 cm^{-1} and 330, 454, 705 cm^{-1} are assigned as carbon out-of-plane bending vibration. Moreover the in-plane carbon bending vibrations may be identified at 359, 519 cm^{-1} in MB and at 355, 595 cm^{-1} in MB.

Present authors assign tentatively the sharp band 258 cm^{-1} and 263 cm^{-1} due to C–$COOCH_3$ an C-$COOCH_2C_6H_5$ out-of-plane bending vibrations fo the MB and BB. Internal vibrations of the subst tuents were not observed due to the limitations of th instrument.

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1. Biswas, D. C., *Ind. J. Phys.*, 1955, **29**, 50
2. Matsuno, K. and Han, K., *Bull. Chem. S* Japan, 1933, **8**, 333.
3. Kohlrausch, K. W. F. and Pongratz, A., *Mona Chem.*, 1933, **63**, 427.
4. Ghosh, J. C. and Kar, B. C., *J. Phys. Ch* 1931, **35**, 1735.
5. Chattopadhyay, S., *Ind. J. Phys.*, 1969, **43**,
6. —, *Ibid.*, 1968, **42**, 335.
7. Whiffen, D. H., *Spectrochim Acta*, 1955, 7,
8. Randle, R. R. and Whiffen, D. H., *Report on Conference Molecular Spectroscopy held London*, October 1954 (Institute of P lium, London), 1955.

THE CRYSTAL STRUCTURE OF TRIGLYCINE CALCIUM IODIDE MONOHYDRATE

THE crystal structures of diglycine calcium chloride tetrahydrate[1] and triglycine calcium bromide[2] were already reported. This is a preliminary note reporting the atomic coordinates in the crystal structure of triglycine calcium iodide monohydrate.

The single crystals of triglycine calcium iodide monohydrate $(NH_2\ CH_2\ COOH)_3\ CaI_2 \cdot H_2O$ were grown from a saturated aqueous solution containing glycine and calcium iodide. The crystal data are as follows: $a = 12 \cdot 07$, $b = 7 \cdot 37$, $c = 19 \cdot 13$ Å, $\beta = 109 \cdot 6°$, $V = 1603 \cdot 2$ Å^3, F.W. $= 537 \cdot 1$ gm, $D_{mea} = 2 \cdot 23$ gm $\cdot$ cm^{-3}, $D_{cal} = 2 \cdot 22$ gm $\cdot$ cm^{-3}, $Z = 4$, μ(Cu K_α) $= 367 \cdot 4$ cm^{-1} and the space group is $P2_1/c$. The density was measured by floatation, in a mixture of bromoform and carbon tetrachloride.

The three-dimensional intensity data (hKl, $K = 0$ to 6, $\mu r = 2 \cdot 33$; hkL, $L = 0$, $\mu r = 2 \cdot 53$) were collected using Cu K_α radiation by the multiple-film equi-inclination Weissenberg technique. The spot-shape, absorption, Lorentz and polarisation corrections were applied in the usual way. From a three-dimensional Patterson synthesis, the positions of the calcium atom and the two iodine atoms were determined.

TABLE I

Fractional atomic coordinates for triglycine calcium iodide monohydrate

Atom	x	y	e
I (1)	0·3654	0·1480	0·1202
I (2)	0·3764	0·1505	0·3696
Ca	0·0223	0·4614	0·2240
O (11)	0·141	0·493	0·156
O (21)	0·183	0·637	0·063
C (11)	0·206	0·593	0·130
C (21)	0·330	0·644	0·185
N (11)	0·365	0·509	0·252
O (12)	0·129	0·824	0·375
O (22)	0·179	0·611	0·311
C (12)	0·199	0·703	0·366
C (22)	0·316	0·672	0·434
N (12)	0·339	0·817	0·490
O (13)	0·023	0·272	0·326
O (23)	−0·025	0·466	0·401
C (13)	0·028	0·325	0·391
C (23)	0·101	0·212	0·458
N (13)	0·048	0·242	0·520
O_w (1)	0·104	0·139	0·227

Thereafter, successive Fourier and difference Fourier syntheses revealed the rest of the structure. Least squares refinement was carried out using the LALS program (originally written by Gantzel, Sparks and Trueblood and modified later by Liminga of Uppsala and also the University of Madras) on an IBM 370/155 computer, at the Indian Institute of Technology, Madras. With anisotropic thermal parameters for the calcium and iodine atoms and isotropic thermal parameters for the other atoms, the residual index at present is 0·117 for 1230 observed reflections. Further refinement is under progress. The present fractional atomic coordinates are presented in Table I.

The calcium atom is coordinated to seven oxygen atoms, two of them belonging to water molecules and the rest to the carboxyl groups of the glycine molecules. The Ca–O distances range from 2·31 to 2·54 Å. No iodine atom coordinates to calcium. The glycine molecules have the normal bond distances and bond angles as found in other similar structures[3]. The hydrogen bonds are between the nitrogen, oxygen and iodine atoms.

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---

1. Natarajan, S. and Mohana Rao, J. K., *Curr. Sci.*, 1976, **45**, 490.
2. — and —, *Ibid.*, 1976, **45**, 793.
3. Mohana Rao, J. K. and Viswamitra, M. A., *Acta Cryst.*, 1972, **28 B**, 1484.

BEST EXPERIMENTAL PARAMETERS OF THE α-HELIX FROM PROTEIN CRYSTALLOGRAPHIC DATA

THE determination of the structures of globular proteins to the atomic detail using X-ray diffraction technique, has, in recent years, reached a good degree of accuracy with the crystallographic R-factor around 25% being quoted[1] and values around 12% for small proteins[2]. The coordinates are accurate to about 0·2 Å in many cases with a higher accuracy of about 0·1 Å being not ruled out for smaller proteins.

New methods and techniques for extracting the wealth of information from structural data have recently been proposed[3,4] and their utility demonstrated[5,6]. These have led to a better visualisation and characterisation of protein chain-folding. In fact, an indirect bonus was the observation of a new secondary structure of protein[7], to which the authors were led from the study of the θ chain plot.

TABLE I

Helical and statistical parameters for H, H_1 and H_3 helices

Helix	t (°)	h (Å)	r_N (Å)	r_C^{α} (Å)	r_C^{1} (Å)	r_O (Å)	$\sigma(\eta)$ (°)	$\sigma(\eta_{ij})$ (°)
H	98·7 (20·7)	1·51 (0·37)	1·62 (0·40)	2·40 (0·62)	1·82 (0·43)	2·15 (0·41)	3·9	10·6
H_1	100·9 (2·4)	1·49 (0·05)	1·56 (0·15)	2·30 (0·14)	1·72 (0·09)	2·04 (0·14)	1·5	1·7
H_2	100·2 (2·2)	1·50 (0·06)	1·54 (0·10)	2·27 (0·10)	1·68 (0·11)	2·00 (0·10)	1·9	2·2
Best Value*	100·5 (2·3)	1·495 (0·055)	1·55 (0·13)	2·28 (0·12)	1·70 (0·10)	2·02 (0·12)		

* Mean of H_1, H_2 helices.

Besides these, the methods could be extended readily to the study of the helical regions in a protein. Details of these, which involve the use of η_{ij} plots, the stereogram and statistical parameters, devised for this purpose, are reported elsewhere[8]. The object of this note is to present one final result that could be arrived at from these applications, namely the calculation, from experimental data, of the "best" values (best in the least-squares sense) of the helical parameters for the α-helix, the data used being those of myoglobin (Perutz, personal communication to Professor R. Srinivasan). It turns out that the best helical regions are to be found in the H-helix. Judged from η_{ij} plot, the stereogram and the statistical parameters for details see ref. 8 this helix shows a clear bend with two sub-helical regions H_1 (residues 133 to 139) and H_2 (residues 142 to 149). The angle between these two subhelices is 11°[2].

With the use of radial and helical projections the least-squares fit for the best helical parameters have been deduced from the two sub-helices and are given in Table I. The values agree with the earlier values of Pauling[11]. As far as the authors are aware the values reported here are the first precise experimental determination from protein crystallographic data of the α-helical parameters.

We wish to thank Dr. M. F. Perutz for providing us with the latest coordinates of myoglobin.

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1. Deisenhofer, J. and Steigeman, N., *Acta Cryst.*, 1975, **B31**, 238.
2. Watenpaugh, K. D., Sieker, L. C., Herriott, J. R., and Jensen, L. H., *Ibid.*, 1973, **B29**, 943.
3. Srinivasan, R. and Balasubramanian, R., *Curr. Sci. (India)*, 1975, **44**, 649.
4. —, — and Rajan, S. S., *J. Mol. Biol.*, 1975, **98**, 739.
5. —, — and —, *J. Theor. Biol.*, 1976 (In press).
6. Rajan, S. S., Balasubramanian, R. and Srinivasan, R., *Indian J. Biochem. Biophys.*, **14**, 14.
7. Srinivasan, R., Balasubramanian, R. and Rajan, S. S., *Science*, 1976, **194**, 720.
8. Rajan, S. S. and Srinivasan, R., *Biopolymers*, 1977 (In press).
9. Klug, A., Crick, F. C. and Wyckoff, H. W., *Acta Cryst.*, 1958, **11**, 199.
10. Fraser, R. D. B. and MacRae, R. P., *Conformation of Proteins and Related Synthetic Polypeptides*, Academic Press, London, 1973, p. 14.
11. Pauling, L., Corey, R. B. and Branson, H. R., *Proc. Nat. Acad. Sci., U.S.*, 1951, 37, 205.

SPECTROPHOTOMETRIC DETERMINATION OF FLUORIDE USING RESACETOPHENONE OXIME AS REAGENT

RESACETOPHENONE OXIME (2, 4-dihydroxy-acetophenone oxime) forms chelates with many metal ions. The reagent is extensively employed in these laboratories for the amperometric, conductometric, and volumetric[1], spectrophotometric[2] and gravimetric[3] determination of many metal ions.

Neelakantam and Kantharaj Urs[4] have employed the oxime for the colorimetric estimation of uranium. The present paper deals with the application of the colour reaction between uranium (VI) and the oxime for the possible determination of fluoride ion. The orange-red colour of the complex is formed from pH 5 to 9. The spectrum in the visible range (400–700 nm) of the complex showed a maximum absorbance at 400 nm. The detailed studies were carried out at 400 nm and at pH 5·9. The colour of the complex decreased with the addition of fluoride. The decrease in the absorbance of the complex is found proportional to the amount of fluoride added.

Reagents

Resacetophenone oxime (mp. 199–200° C) was prepared in the laboratory by the standard procedure[5,6] and purified by recrystallisation from hot water. A solution of the oxime (0·1 M) was prepared in 50% aqueous ethanol. Stock solutions (0·01 M) of uranyl acetate and fluoride were used in this investigation.

ELICO instrument SPECTROCOL Model CL 23 was employed for all absorbance measurements.

Recommended Procedure

To 1·5 ml of uranium solution taken in a 25 ml measuring flask, 2 ml of acetate buffer (pH 5·9) and 2 ml of oxime solution were added. To this, known amounts of the standard fluoride solution were added and the contents made upto the mark with distilled water. The absorbance of the solutions was measured at 400 nm against distilled water as blank. The data obtained showed that the absorbance is proportional to the amount of fluoride (Fig. 1), indicating the suitability of the reaction for the determination of fluoride.

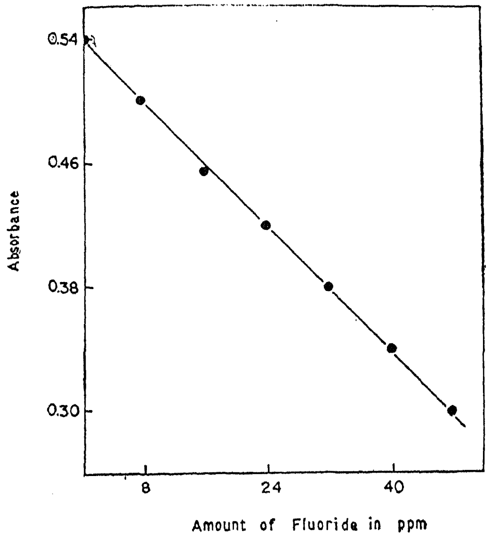

FIG. 1. Plot of amount of fluoride *versus* absorbance.

The colour formation of the complex is rapid and the absorbance of the complex is stable for more than 72 hours. The standard deviation is 0·015. The molar absorptivity and the Sandell sensitivity[7] are 900 litre mole^{-1} cm^{-1} and 0·265 μg/cm^2 respectively.

Interference

Br$^-$, Cl$^-$ and NO$_3{}^-$ did not interfere in the determination even when present in large amounts. Iron(III), aluminium(III), citrate, oxalate, carbonate, bicarbonate, phosphate interfere. SO$_4{}^{--}$ interferes if the amount is greater than 3,500 ppm.

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---

1. Krishna Reddy, Y., *Ph.D. Thesis,* Sri Venkateswara University, India, 1969.
2. Neelakantam, K. and Sitaraman, M. V.; *Curr. Sci.,* 1945, 14, 320.
3. — and Raju, N. A., *Ibid.,* 1958, 19, 383.
4. — and Kantharaj Urs, *J. Sci. Ind. Res.,* 1952, 11 B, 79.
5. Arthur I. Vogel, *A Text Book of Practical Organic Chemistry,* 1948, p. 341.
6. Dey, B. B. and Sitaraman, M. V., *Laboratory Manual of Organic Chemistry,* 1952, p. 264.
7. Sandell, E. B., *Colorimetric Determination of Trace of Metals,* 3rd Ed., Interscience, New York, 1959.

A FACILE ONE-STEP SYNTHESIS OF 2-ARYL AND HETERYL-1, 8-NAPHTHYRIDINES

SEVERAL 1, 8-naphthyridines have been found to possess diverse types of biological activities including bacteriocidal[1] and antimalarial[2] properties. It has also been known that 1-ethyl-3-carboxy-7-methyl-1, 8-napththyridin-4-one (nalidixic acid) and its derivatives are particularly effective against gram-negative bacteria[3]. Taking into account the varied activities exhibited by 1, 8-naphthyridine system, a series of 2-aryl and heteryl 1, 8-naphthyridines (II) have been made in the present investigation, with a view to see the effect of aryl and heteryl moieties on the biological activity of naphthyridine molecule.

2-Phenyl-1, 8-naphthyridine was earlier synthesized by Hawes and Wibberley[4] by condensing 2-aminonicotinaldehyde with benzoylacetonitrile to afford 2-phenyl-3-cyano-1, 8-naphthyridine. The cyano group was then removed by first hydrolysing it to carboxyl under alkaline conditions and subsequent thermal decarboxylation. Except for this, there seems to be no other reference available in literature on the synthesis of the title compounds.

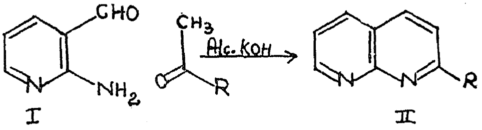
The present communication deals with a facile one-step synthesis of 2-aryl and heteryl-1, 8-naphthyridines unlike Hawes and Wibberley's (*loc. cit.*) procedure which consisted of three steps. This method involves the direct condensation of 2-aminonicotinaldehyde (I) with appropriate aryl methyl ketone in the presence of few drops of 20% alcoholic potassium hydroxide using ethanol as solvent. The progress of the reaction was observed after every hour on TLC using benzene as solvent system, and refluxing was stopped when

TABLE I

2 *Aryl and heteryl*-1, 8-*naphthyridines (II)*

Sl. No.	1-8-Naphthyridine	mp (0° C)	Yield %	Solvent	Mol. Formula	Analysis Calculated C			Found C	H	N
						C	H	N	C	H	N
1.	2 Phenyl	116[4]	80	Cyclohexane	.	..	..	..	..	..	[illegible]
2.	2 *p*-Methylphenyl	147	75	Cyclohexane	$C_{15}H\ N_2$	81·83	5·45	12·72	81·61	5·41	[illegible]
3.	2 *p*-Methoxyphenyl	148	72	Benzene	$C_{15}H_{12}N_2O$	76·28	5·08	11·87	76·37	5·13	[illegible]
4.	2 *p*-Hydroxyphenyl	254	78	Ethylacetate	$C_{14}H_{10}N_2O$	75·65	4·50	12·61	75·27	4·51	[illegible]
5.	2-*o*-Hydroxyphenyl	188	76	Benzene	$C_{14}H_{10}N_2O$	75·65	4·50	12·61	75·43	4·52	[illegible]
6.	2-*p*-Nitrophenyl	263	82	Chloroform	$C_{14}H_9N_3O_2$	66·92	3·58	16·73	67·21	3·60	[illegible]
7.	2-*m*-Nitrophenyl	219	80	Ethanol	$C_{14}H_9N_3O_2$	66·92	3·58	16·73	67·17	3·58	[illegible]
8.	2-*p*-Bromophenyl	217	76	Benzene	$C_{14}H_9BrN_2$	58·96	3·15	9·83	60·00	3·21	[illegible]
9.	2-*p*-Chlorophenyl	202	75	Ethanol	$C_{14}H_9ClN_2$	70·00	3·75	11·67	69·77	3·81	[illegible]
10.	2-(α-Naphthyl)	134	78	Benzene/Pet. Ether	$C_{18}H_{12}N_2$	84·39	4·69	10·94	85·01	4·74	[illegible]
11.	2-(3-Pyridyl)	142	71	Benzene	$C_{13}H_9N_3$	75·36	4·34	20·29	75·04	4·09	[illegible]
12.	2-(2-Furyl)	146	68	Pet. ether	$C_{12}H_8N_2O$	73·47	4·08	14·29	74·04	4·11	[illegible]
13.	2-(2-Thienyl)	133	65	Benzene	$C_{12}H_8N_2S$	67·94	3·77	13·20	68·10	3·79	[illegible]

most of the starting material had reacted (2–3 hours). The corresponding 2-heteryl-1, 8-naphthyridines have been obtained in good yields, when heterylmethyl ketones were employed in the place of aryl methyl ketone. Heteryl substituents like pyridyl, furyl and thienyl were selected in view of their biological importance. The purity and homogeneity of these compounds was checked by TLC using benzene-methanol (95:5) as solvent system.



R= Aryl or Heteryl.

All the compounds prepared are listed in Table I along with the m.p., yield, solvent of crystallisation and analytical data.

Finally the 2-phenyl-1, 8-naphthyridine was prepared by the procedure reported earlier[4] and was found to be identical in all respects (m.p., mmp and TLC) with our compound.

The mass spectrum of one representative in the series, *viz.*, 2-*p*-chlorophenyl-1, 8-naphthyridine shows the molecular ion at m/e 240, thus supporting the structure. The NMR spectrum also supports the structural assignment.

The bacteriocidal, insecticidal and other activities which are under study will be reported later along with detailed spectral data.

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---

1. Cilag Ltd., *Swiss Patent*, 1949, 263, 148; *C*
Abstr., 1950, **44**, 6099.
Richter, C., *U.S. Patent*, 2, 517, 929; *Chem. A*
1951, **45**, 672.
Albert, A. and Hampton, H., *Sec. Lectur Bi*
Univ. Coll. London, 1958, p. 96; *Chem. A*
1958, **52**. 8281.
2. Ito, R., Hashimoto, Y, Iida, M. and Haman
Symp. Enzyme Chem., (*Tokyo*), 1950, 4.
Chem. Abstr., 1951, **45**, 7166.
3. Paudler, W. W. and Kress, T. J., In *Adv*
Heterocyclic Chemistry (Katritzky and
ton, ed.), Vol. 11, Academic Press; New
1970, p. 170.
4. Hawes, E. M. and Wibberley, D. G., *J. C*
Soc. (*C*), 1967, p. 1564.

EFFECT OF β AND γ ISOMERS OF HEXACHLOROCYCLOHEXANE ON SOME LIVER AND KIDNEY ENZYMES IN ALBINO RATS

AMONG the various stereo isomers of hexachlor hexane (BHC), the γ-isomer has both strong insect action[1] and high mammalian acute toxicity[2]. β- on the other hand, shows high chronic toxicity of its long persistence and poor elimination[3], and causes a hazard. α- and γ-isomers are known to act as central nervous stimulants, whereas β- and δ-isomers are depressants[4]. The convulsive effect of γ-BHC and narcotic effect of β-BHC in rats have been discussed by Doisy and Bocklage[5]. Effect of acute and long term feeding of technical BHC on several enzyme systems of serum, liver and small intestine in rats have been reported[6–9]. Effect of acute and long term feeding of γ-BHC on serum levels of aminotransferases, lactate dehydrogenase, aldolase and glucose-6-phosphatase have been described[10–11]. γ-BHC is known to stimulate greater activity of the microsomal enzymes of the liver[12]. No detailed reports are available about the biochemical effects produced by β-BHC. Preliminary experiments have shown that β-BHC at 800 p.p.m. level in the diet produced severe toxicity symptoms and mortality during 3rd and 4th weeks. In the present investigation the biochemical effects caused by feeding β- or γ-BHC for 2–3 weeks were studied with reference to some metabolically important enzymes of liver and kidney tissues and reported.

Weanling male rats weighing 45–50 g were fed with diets containing 800 p.p.m. of β-BHC or γ-BHC for 14–18 days and compared with the controls. The basal diet contained (g/100 g) casein, 18; groundnut oil, 10; corn starch, 45·1; cane sugar, 20; mineral salts, 4; B-complex vitamin mixture, 2·5; choline chloride, 0·4; and fat soluble vitamins as solution in groundnut oil. Pure grade BHC isomers obtained from K and K Laboratories, USA, were dissolved in warm groundnut oil and mixed with the basal diet. The rats were observed for two weeks and sacrificed during the third week. The liver and kidney tissues were excised and homogenized in 0·25 M sucrose, and used for enzyme analysis. Alanine and aspartate 2-oxoglutarate aminotransferase activities (EC 2.6.1.2 and EC 2.6.1.1) abbreviated as alanine and aspartate aminotransferase, were determined by the method of Tonhazy *et al.*[13]. Alkaline and acid phosphatase (EC 3.1.3.1 and EC 3.1.3.2) activities were assayed by the method of Hubscher *et al.*[14]. Glucose-6-phosphatase (EC 3.1.3.9) was assayed by the method of Harper[15], while glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was assayed by the spectrophotometric method described by Lohr and Waller[16]. Protein was determined by the method of Lowry *et al.*,[17] and statistical evaluations were done employing Student's *t* test[18].

β-BHC produced no growth retardation during the first two weeks. These rats were sacrificed during the third week (15 and 18th days) as they showed severe toxicity symptoms culminating in coma and death toward the end of the third week. On the other hand, animals treated with γ-BHC had initial growth retardation and loss of appetite during the first five days. Afterwards they regained normal appetite and growth rate comparable to the control animals. This state continued till the 3rd week when they were sacrificed. All animals in the control and β or γ-BHC fed groups weighed between 90 and 100 g at the time of sacrifice.

The various enzyme activities of liver and kidney tissues in experimental rats as well as in controls are given in Table I. In the β-BHC fed rats, an increase in hepatic alanine aminotransferase by about 80%

TABLE I

Effect of BHC isomers on various enzyne activities

Enzyme	Tissue homogenate	Activity			Statistical significance (P)	
		Control (A)	β-BHC (B)	γ-BHC (C)	A∼B	A∼C
Alanine amino-transferase[a]	Liver	66·01±0·68 (12)	118·48±9·1 (10)	98·94±6·73 (9)	<0·001	<0·001
	Kidney	29·90±0·57 (7)	19·19±0·69 (10)	28·67±0·89 (7)	<0·001	N.S.
Aspartate amino-transferase[a]	Liver	66·23±0·85 (12)	46·39±1·36 (10)	45·62±2·33 (9)	<0·001	<0·001
	Kidney	67·69±2·27 (7)	63·06±2·19 (10)	72·93±1·36 (7)	N.S.	N.S.
Alkaline phosphatase[b]	Liver	0·221±0·003 (12)	0·120±0·007 (12)	0·144±0·009 (12)	<0·001	<0·001
	Kidney	13·83±0·27 (5)	31·31±1·65 (10)	14·89±0·58 (7)	<0·001	N.S.
Acid phosphatase[b]	Liver	0·824±0·007 (12)	0·493±0·025 (16)	0·520±0·036 (12)	<0·001	<0·001
	Kidney	0·711±0·004 (5)	0·961±0·079 (9)	1·077±0·099 (9)	<0·1	<0·005
Glucose-6-phosphatase[b]	Liver	6·83±0·10 (9)	4·77±0·34 (9)	11·46±1·18 (6)	<0·001	<0·01
Glucose-6-phosphate dehydrogenase[c]	Liver	0·189±0·003 (10)	0·433±0·035 (16)	0·262±0·028 (8)	<0·001	<0·02

All values are means ± S.E.M. with number of rats in parenthesis.

a = micrograms of pyruvate released/10 min/mg protein; *b* = micromoles of P_i released/hr/mg protein; = △ E/min/mg protein.

and a decrease in hepatic aspartate aminotransferase by about 30% were observed, while kidney level of alanine aminotransferase decreased by about 35% in comparison with the controls. The alkaline and acid phosphatases of liver in β-BHC fed rats were reduced by about 45% and 40% respectively, while the kidney enzymes increased by about 125% and 35% respectively. Liver glucose-6-phosphatase activity was decreased by about 30% in β-BHC fed rats while glucose-6-phosphate dehydrogenase activity was increased by as much as 130%. All these effects of β-BHC on the enzyme levels are statistically significant.

In the γ-BHC fed rats, an increase in hepatic alanine aminotransferase by about 50% and a decrease in hepatic aspartate aminotransferase by about 30% were observed, while no significant effects on these enzymes occurred in kidneys. Alkaline and acid phosphatases of liver in γ-BHC fed rats were decreased by about 35% each, while only an increase of kidney acid phosphatase by about 40% occurred. Both glucose-6-phosphatase and glucose-6-phosphate dehydrogenase levels of liver in γ-BHC fed rats were increased by about 65% and 40% respectively.

These effects of BHC isomers on liver and kidney enzymes have not been reported. The observed effects on these metabolically important enzymes of liver and kidney imply that both the liver and kidney get affected during the continued intake of β-BHC. Only liver seems to be involved under the experimental conditions in γ-BHC fed animals.

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---

1. Slade, R. E., *Chem. and Indus.*, 1945, p. 314
2. Lehman, A. J., *Assoc. Food and Drug Officials U.S. Quart..Bull.*, 1951, **15**, 122.
3. Davidow, B. and Frawley, J. P., *Proc. Soc. Exptl. Biol. and Med.*, 1951, **76**, 780.
4. McNamara, B. P. and Krop, S., *J. Pharmacol. and Exptl. Therap.*, 1948, **92**, 147.
5. Doisy, E. A. and Bocklage, B. C., *Proc. Soc. Exptl. Biol. and Med.*, 1950, **74**, 613.
6. Sasinovich, L. M., Alekhina, S. M. and Odintsova, I. L., *Vrach Delo.*, 1974, **10**, 133; *Chem. Abs.*, 1975, **82**, 93915.
7. Zakirov, U. B., Volokhvyanskii, E. A. and Kadyrov, U. Z., *Pharmakol. Toksikol.*, 1974, **37**, 593; *Chem. Abs.*, 1975, **82**, 81422.
8. Starek, Andrej, *Med. Pracy.*, 1970, **21**, **554**; *Chem. Abs.*, 1971, **74**, 97226.
9. Kashyap, S. K., Gupta, S. K., Venkatakrishna Bhatt, H. and Shah, M. P., *Ind. J. Med. Res.*, 1976, **64**, 768.
10. Alekhina, S. M., *Toksikol. Pestets. Klin. Otravl.* 1971, **9**, 150; *Chem. Abs.*, 1972, 77, 148203
11. Bronish, G. A., Rusetskii, V. and Vysotskaya, B., *Gigiena i Toksikol. Novykh Pestitsidov i Klinika Otravlenii.*, 1962, **268**; *Chem. Abs.*, 1964, 60, 2240.
12. Conney, A. H., *Pharmacol. Revs.*, 1967, **19**, 317.
13. Tonhazy, N. E., White, N. E. and Umbreit, W. W., *Arch. Biochem. Biophys.*, 1950, **28**, 36.
14. Hubscher, G. and West, G. R., *Nature*, 1965, **205**, 799.
15. Harper, A. E., *Methods of Enzymatic Analysis*. Edited by H. V. Bergmeyer, Acad. Press, N.Y. 1963, p. 788.
16. Lohr, G. W. and Waller, H. D., *Ibid.*, 1963, p. 744.
17. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
18. Snedecor, G. W. and Cochran, *Statistical Methods*. The Iowa State Univ. Press, Iowa, 1967, p. 100.

A NEW SYNTHESIS OF DICOUMAROLS

WITH a general aim to synthesise coumarins containing fused ring systems in 3:4 positions, the condensation reaction between 4-hydroxy coumarins and Schiff's base has been investigated. It has earlier been reported from these laboratories that the Schiff's bases function as potential sources of aldehydes in a number of condensation reactions[1].

In the present investigation, 6-methyl-4-hydroxy coumarin was condensed with the Schiff's base from benzaldehyde and aniline, in acetic acid at room temperature (Fig. 1). The reaction was instantaneous and exothermic. The product obtained had mp 228° C and showed a single spot on T.L.C. in benzene/ethyl acetate (1:1). The compound was soluble in 5% sodium bicarbonate solution indicating that the hydroxy group in 4-position was free and not involved in cyclisation. Further, the compound did not contain nitrogen as indicated by sodium fusion test.

Suspecting it to be of a dicoumarol type, an authentic sample was prepared from 6-methyl-4-hydroxy coumarin following Sullivan *et al*[2]. procedure (m.p. 227° C). Both these compounds were found to be identical in all respects (mmp and superimposable IR). Thus, the compound obtained by the above condensation is 3, 3'-benzylidine bis-6-methyl-4-hydroxy coumarin.

Following this procedure, dicoumarols of the type have been prepared in good yields from the following coumarins namely, simple 4-hydroxy coumarin, 7-methyl-4-hydroxy coumarin, 8-methyl-4-hydroxy coumarin, 6-chloro-4-hydroxy coumarin, 8-chloro-4-hydroxy coumarin, 6-chloro-7-methyl-4-hydroxy coumarin, 6, 8-dichloro-4-hydroxy coumarin. The compounds obtained have been given in Table I along with their mps and the frequency of carbonyl absorption in IR.

TABLE I

Dicoumarols with mp and IR data

Name of the substituent	m.p. C°	% yield	Carbonyl frequency in IR (cm⁻¹)
Simple	208[2]	80	1660
6-methyl	228[3]	80	1665
7-methyl	235	80	1660
8-methyl	262	80	1660
6-chloro	220[4]	80	1665
6-chloro-7-methyl	263[5] (lit m.p. 214–6)	75	1665
6, 8-dichloro	255	75	1670

This method does not seem to have been reported earlier for the synthesis of dicoumarols. The reaction conditions are fairly simple and yields are quite good in all cases. This condensation may prove useful for the synthesis of dicoumarols from sensitive aldehydes.

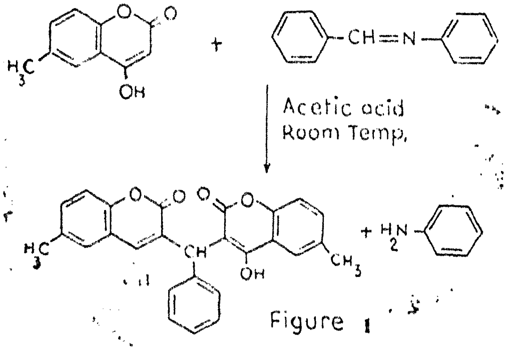

Figure 1

Experimental

General Procedure. 4-hydroxy coumarin (0·02 mole) and Schiff's base (from benzaldehyde and aniline) (0·01 mole) were mixed in acetic acid (20 ml). The clear dark brown solution obtained was shaken well for about 15 minutes at room temperature. A colourless substance was precipitated immediately. This was filtered, dried and recrystallised from methanol as colourless crystals.

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1. Veeranagaiah, V., Ratnam, C. V. and Subba Rao, N. V., *Indian J. Chem.*, 1972, **10**, 133.
2. Sullivan, W. R., Heubner, C. F., Stahmann, M. A. and Link, K. P., *J. Am. Chem. Soc.*, 1943, **65**, 2288.
3. Josef Klosa; *Arch. Pharm.*, 1956, **289**, 156.
4. —, *Ibid.*, 1956, **289**, 143.
5. —, *Ibid.*, 1956, **289**, 240.

INTERFACIAL TENSION AND PARTICLE SIZE OF NONAQUEOUS EMULSIONS

IN continuation of earlier work on oil-in-oil emulsions[1–3], the present communication describes the effect of temperature on the interfacial tension of oil-oil interface in presence of an emulsifier and an effort has been made to correlate the interfacial tension with the mean particle size and the stability of the resulting emulsions.

Monochlorobenzene and ethylene glycol were employed as the two nonaqueous phases and the surfactants used were (*a*) polyoxyethylene sorbitan monostearate (Tween 60; KL), (*b*) polyoxyethylene sorbitan monooleate (Tween 80; KL), and (*c*) polyoxyethylene sorbitan monolaurate (Tween 20; KL). The oil-in-oil emulsions were prepared with 1:1 phase ratio by volume and 0·1% concentration of nonionic surfactant (*w*/*v* % of emulsion). The mixtures were emulsified with the help of Braun emulsator.

The interfacial tension of monochlorobenzene-ethylene glycol interface with the surfactant was determined by the drop volume method[4]. Three or more runs were made for each measurement. The interfacial tension ($O_1 \gamma O_2$) at the oil (O_1)-oil (O_2) interface in presence of surfactant was calculated from the expression.

$$ {}_{O_1}\gamma_{O_2} = \frac{v\,(\rho_1 - \rho_2)g}{2\pi r \phi (r/v^{1/3})} = \left[\frac{v\,(\rho_1 - \rho_2)g}{r}\right] F \qquad (1)$$

where v is the drop volume, r is the radius of the capillary tip, ρ_1 and ρ_2 are the densities of the respective phases and F, the correction function, depending on v/r^3, is derived from the drop-volume correction table.

Particle size analyses of nonaqueous emulsions were carried out by photomicrographic method[5]. Immediately after the preparation of an emulsion, the mean globule diameter (Dm) was determined. Further determinations were made at different temperatures in the range of 15°–60° C. Dm was calculated from the relationship[6]

$$ \mathrm{Dm} = \left\{\frac{n_1 D_1^3 + n_2 D_2^3 + \ldots + n_n D_n^3}{n_1 + n_2 + \ldots + n_n}\right\}^{1/3} = \left\{\frac{\Sigma n D^3}{\Sigma n}\right\}^{1/3} \qquad (2)$$

where n_1, n_2, ... n_n are the number of globules with diameters D_1, D_2, ... D_n, respectively, Figure 1 shows the influence of temperature on the interfacial tension of monochlorobenzene-ethylene glycol interface in the presence of three different non-ionic surfactants. In all cases, there is an increase in the interfacial tension of oil-surfactant-oil interface with an increase in temperature. Under similar conditions, when a surfactant was added to monochlorobenzene phase, the values of interfacial tension have been found to be slightly lower than when the surfactant was dissolved in ethylene glycol phase.

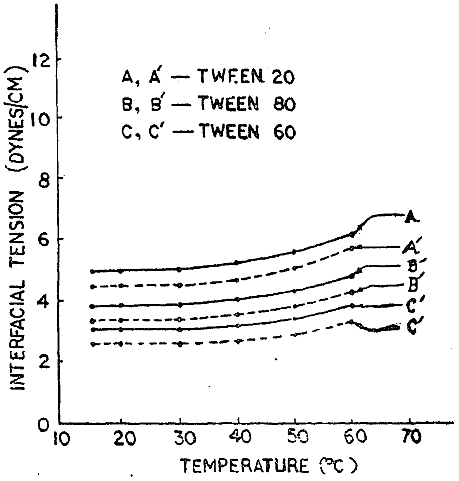

FIG. 1. Effect of temperature on the interfacial tension of monochlorobenzene-ethylene glycol interface in the presence of surfactant: —— surfactant dissolved in ethylene glycol, ... surfactant dissolved in monochlorobenzene.

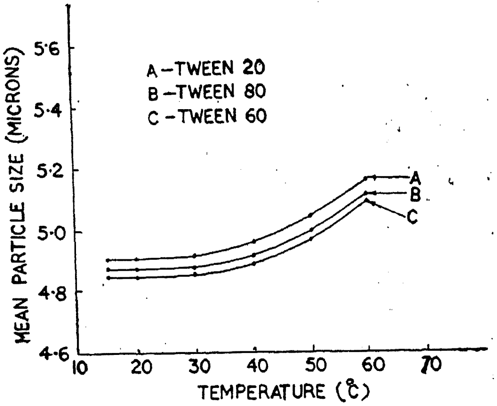

FIG. 2. Effect of temperature on the mean particle size of oil-in-oil emulsions stabilized with surfactants.

The effect of temperature on the mean particle size of oil-in-oil emulsions stabilized with three different nonionic surfactants is shown in Fig. 2. There is an increase in the mean particle size of emulsions with an increase in temperature of emulsions. As compared to higher temperatures, particle size of emulsions increases much more slowly at lower temperatures, during the course of increasing the temperature of emulsions, because the rate of coalescence of emulsion droplets appears to be slow and the viscosity of the medium is higher at lower temperatures.

It is apparent from these studies that the interfacial tension increases with increasing temperature and so do the particle size. It is well known that the stability of emulsions is related to the particle size of emulsion droplets *i.e.*, the smaller the particle size, more stable are the emulsions. Thus it may be concluded from the interfacial tension data that the stability of oil-in-oil emulsions decreases with an increase in temperature of emulsions from 15°–60° C and as compared to higher temperatures, the stability of the emulsions decreases much more slowly at lower temperatures, because the stability of the emulsions is more at lower temperatures.

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1. Sharma, M. K., *J. Colloid Interface Sci.*, 1975, **53**, 340.
2. —, *Curr. Sci.*, 1975, **44**, 770.
3. —, *Ibid.*, 1977, **46**, 131.
4. Harkins, W. D. and Brown, F. E., *J. Am. Chem. Soc.*, 1919, **41**, 499.
5. Shillaber, C. P., *Photomicrography in Theory and Practice*, Wiley, New York, 1949, p. 41.
6. Bezemer, C. and Schwarz, N., *Kolloid-Z.*, 1956, **146**, 145.

MOTHER-CHILD INCOMPATIBILITY IN THE ABO SYSTEM

SELECTION is known to exist because of maternal-fetal antigenic incompatibilities with regards to the ABO system and the expected distortions in genetic ratios have already been demonstrated.[1-4]

In order to investigate the selective mechanism operating on ABO bloodgroups an investigation was carried out on some village populations of coastal Andhra Pradesh. Samples from 1021 couples, with wives ranging in age from 20 to 40 years were collected and a total of 4144 children could thus be tested with ABO bloodgroups. The results are presented in Table I.

There appears to be no striking difference in the proportion of childless couples between the two groups ($\chi_1^2 = 0\cdot01$). The mean number of living children between the two matings presents a significant value ($t = 5\cdot510$; $P < 0\cdot001$). As to segregation ratio of the bloodgroups of children, comparisons between

TABLE I

Data of fertility of ABO compatible and incompatible matings

Observations	Compatible Matings $n = 589$	Incompatible Matings $n = 432$
1. Mean number of living children per mating	4·51	3·73
	($t = 5·510$;	$P < 0·001$)
2. Proportion of childless marriages	0·031	0·027
	($\chi_1^2 = 0·01$)	
3. Mean number of pregnancies excluding abortions	5·26	4·90
4. Proportion of Pre-natal Mortality of children	0·348	0·476
5. Proportion of Post-natal mortality of children	0·411	0·694
6. Fetal wastage	17·15%	31·90%

♀A × ♂O and ♂A × ♀O matings revealed an apparent increase of 0 children from ♀O× ♂A matings than from ♀A × ♂O matings, which are significant at 5 % level ($\chi_1^2 = 5·31$). There is 21% deficiency of "A" children in ♀O × ♂A as compared to ♀A × ♂O matings. The incompatibility matings have more "observed" values than "expected" under random matings. The Chi-square value is again significant at 5% ($\chi_1^2 = 4·69$), in respect of the comparison between the ♀B × ♂O and ♂B × ♀O. There is a 16% deficiency of "B" children in ♀O × ♂B matings as compared to ♀B × ♂O matings.

TABLE II

Frequencies of pre- and post-natal deaths in ♀A× ♂O/♀O × ♂A and ♀B × ♂O/♀O × ♂B matings

Sl. No.	Matings	No. of Live	No. of Deaths		X^2
	♀ × ♂	Births	Pre Natal	Post Natal	
1.	A × O	464	41	40	2·436
2.	O × A	401	50	68	1·060
3.	B × O	462	42	38	1·457
4.	O × B	402	58	73	4·728*

* P < 0–02

The differences between the matings as mentioned above excepting sl. no. 4 are significant only at 5 %, while the segregation of children out of ♀A × ♂B do not show any significant value indicating that there is no deficit of incompatible children.

Although extremely important as a health problem, ABO erythroblastosis does not represent a serious fetal loss from the biological point of view, while evidence on many kinds shows a much larger and biologically important loss of possible incompatible children early in pregnancy/prior to the recognition of pregnancy. The only definite evidence for selection was that against incompatible fetuses or neonates in the ABO system due to hemolytic disease. New studies about other causes of incompatibility selection are needed to help in the evaluation of its importance in man.

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---

1. Matsunaga, E., *Blut*, 1956, **2**, 188.
2. Cohen, B. H., *Amer. J. Hum. Genet.*, 1960, **12**, 180.
3. Newcombe, H. B., *Ibid.*, 1963, **15**, 449.
4. Chakravartti, M. R., *Indian J. Phy. Anth. Hum. Gen.*, 1976, **1**, 128.

DETECTION OF ANTIGENS AND ANTIBODIES IN FILARIAL SERA BY COUNTER CURRENT IMMUNOELECTROPHORESIS

THE importance of counter current immunoelectrophoresis (CIE) as a good serological technique is being recognized by various investigators, for its simplicity and sensitivity with added advantage of detection of antigen and antibody in the sample at same time. Desowitz and Una[1] (1976) have shown the presence of antibodies to microfilarial and adult male *D. immitis* antigens in human and animal filarial sera by counter current immunoelectrophoresis.

W. bancrofti microfilariae were isolated from blood samples from human carriers by dextran sedimentation and antisera were raised in rabbits for the microfilarial antigen. Anti microfilarial sera and microfilarial antigen were used for detection of antigen and antibody in the sera of humans infected with *W. bancrofti* by double diffusion and counter current immunoelectrophoresis.

Sixteen sera from human carriers (microfilarial count ranging from 1 to 94/20 c.mm. of blood) were analyzed. No precipitin band was observed either for the antigen or antisera by double diffusion. However counter current immunoelectrophoresis of human filarial sera showed the presence of precipitin bands (1 to 3) with rabbit antimicrofilarial sera indicating the presence of soluble antigens in all the cases and one precipitin band with microfilarial antigen indicating the presence of antibody in two cases. Use of counter current immunoelectrophoresis in detection of soluble antigens is being investigated further for application as additional confirmatory test in the diagnosis of filariasis.

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---

1. Desowitz, R. S. and Una, S. R., *J. Helminthology*, 1976, 50, 53.

COMPARISON OF THE PROTEIN COMPONENTS OF HEN'S EGG YOLK LOW DENSITY FRACTION OF GRANULES (LDFG) AND LOW DENSITY FRACTION (LDF)

LDFG AND LDF (also called very low density lipoprotein; VLDL) of hen's egg yolk have long been considered to be similar because of their similarity in lipid content and lipid composition[1,2]. Recently, the protein components of VLDL were isolated and characterised in this laboratory[3]. Here we report preliminary data to show that the protein components of LDFG and VLDL are similar.

LDFG of white plymouth rock egg was carefully separated from granule solution after ultracentrifugation[4]. It was dissolved in 0·05 M Tris-HCl buffer pH 8·2 and dialysed against the same buffer with three to four changes. The solution was centriguged to remove precipitated lipovitellin-phosvitin complex and was chromatographed on DEAE-cellulose column as described by Raju and Mahadevan[3]. LDFG-apoprotein was obtained from LDFG and fractionated using Sephadex G-200 column[5]. SDS-polyacrylamide gel electrophoresis was performed by the method of Weber and Osborn[6].

Sephadex G-200 elution pattern of LDFG-apoprotein consists of three fractions namely, zone A, zone B and zone C. These three fractions resemble those of VLDL-approprotein shown in Fig. 1. Variation in the distribution of zone C-protein fraction in LDFG and VLDL is evident from Fig. 1. This differences in ratio of zone C to zone A protein fractions could be due to a difference in the distribution of zone C-protein fraction or due to the difference in the two egg strains (VLDL is obtained from white leg horn egg). Figure 2 shows similarities in SDS-gel pattern of the apoproteins of LDFG and VLDL. Both these experiments suggests that the protein components of LDFG and VLDL are similar.

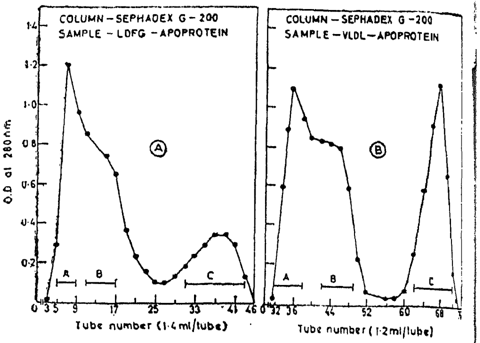

FIG. 1. A: Gel filtration of LDFG-apoprotein on Sephadex G-200. About 60 mg protein dissolved in 2·8 ml of 0·5% SDS was loaded on Sephadex G-200 column (1·8 cm × 73 cm) and the column was eluted with 0·5% SDS. 1·4 ml fractions were collected at a flow rate of 15 ml/hour. B: Gel filtration of VLDL-apoprotein on Sephadex G-200[5]. About 50 mg protein loaded in 1 ml of 0·5% SDS on sephadex G-200 column (1·3 × 100 cm) and the column was eluted with 0·5% SDS. 1·2 ml fractions were collected at a flow rate of 6 ml/hr.

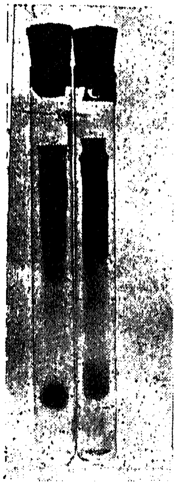

FIG. 2. SDS-polyacrylamide gel electrophoresis of the apoproteins of VLDL and LDFG.

Picture : VLDL and LDFG.

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---

1. Martin, W. G., Tattrie, N. H. and Cook, W. H., *Can. J. Biochem. Physiol.*, 1963, **41**, 657.
2. Cook, W. H. and Martin, W. G., *Structural and Functional Aspects of Lipoproteins in Living Systems*, (Ed.) Tria, E. and Scanu, A. M. Academic Press, London and New York, 1969, p. 579.
3. Raju, K. S. and Mahadevan, S. *Anal. Biochem.*, 1974, **61**, 538.
4. Wallace, R. A., *Ibid.*, 1965, **11**, 297.
5. Raju, K. S. and Mahadevan, S., *Biochim. Biophys. Acta*, 1976, **446**, 387.
6. Weber, K. and Osborn, M., *J. Biol. Chem.*, 1969, **244**, 4406.

A BRIEF NOTE ON THE GEOLOGY AND OCCURRENCE OF URANIUM AT BODAL, DISTRICT RAJNANDGAON (M.P.)

In the course of radiometric survey in the year 1973 in parts of Rajnandgaon District (M.P.)., moderately high radioactivity was discovered by the author in hornblende-quartz-biotite rocks—an uncommon host for uranium—exposed SW of Bodal village. Some of the surface samples have assayed more than 0·1% U 308 (Chem).

The outcrops showing uranium mineralisation exhibit shades of dark green colour with reddish-brown and yellowish encrustations of hydrated oxides of iron. They are generally fine-grained, with occasional porphyritic texture and are composed of hornblende, quartz and biotite in a groundmass of fine-grained equigranular quartz, Pyrite, ilmenite, Fe-oxides, occasional feldspar and calcite occur as accessories. Hornblends occurs in prismatic and acicular forms and sometimes in resette clusters. Under the microscope, hornblende is seen to alter to biotite and chlorite and the released calcite and iron oxides permeate into the microfractures. Wherever the rock is mineralised, the black oxide of uranium is found to be associated with the iron oxides. Quartz is often brecciated and crushed and the microfractures as well as the intergranular spaces are often occupied by veins of hornblende quartz and pennenite.

In the area under consideration, the radioactive hornblende-quartz-biotite rocks form a part of the geological setting comprising—(*a*) Ferruginous rhyolitic rocks together with quartz porphyry—their hypabasal equivalents. The former is profusely intruded by quartz veins containirg frequent shows of Pb–Cu mineralisation, as in Karamtara, 8 km. NE and Thelkhadand, 6 Km. south of Bodal. (*b*) Basic rock complex occurring as extensive massive bodies or huge dykes cutting across the quartz porphyry and ferruginous rhyolitic rocks and chiefly made up of pyreboles, biotite and Ca-plagioclase. (*c*) Light coloured biotite granites, which are presumably the southward continuation of Dongragarh granites, occurring in the southern and western margins of the area.

The basic rock complex shows frequent textural and compositional variations and the uraniferous hornblende-quartz-biotite rocks are by far the most conspicuous and easily discernible member of this complex. They display numerous vertical to subvertical joints and shear fractures, the most dominant shear direction being N40° W. This shear-zone is characterized by the presence of brecciated and recrystallised quartz and is marked by the crude alignment of the lath-shaped minerals and moderately high radioactivity.

From the field and laboratory investigations of various rock types it appears that the radioactive hornblende quartz-biotite rocks are a hybrid product resulting from partial melting and assimilation of quartz porphyry by some basic intrusion. This view is supported by the presence of xenoliths of the latter enclosed in these rocks as well as traces of poorly assimilated rocks found sporadically in the area.

It is suggested that the origin of uranium and other polymetallic mineralisation may be associated with the late hydrothermal phase related to granitic intrusions along the main N 40° W shear zone, during orogenic cycles in the post-Amgaon period. The evidence of hydrothermal activity is afforded by the presence of criss-crossing quartz veins, appearance of chlorite, biotite and pennenite veinlets and ferrugination of the rocks, especially along the shear zone.

Detailed radiometric survey indicated that the surface manifestation of radioactivity can be followed along N 40° W trend intermittently for a strike length of 750m. Further, to the north-west and south-east, the rocks go under thick soil cover. Radon emanation survey was undertaken to investigate the soil-covered area. In the north-western soil-covered portion of the area, the radon values in a few patches exceed 200 times the background value, forming a 300 m. long amonalous zone in the N 40° W direction.

Sub-surface exploration by borehole drilling is in progress for proving the potentiality of this deposit. Results so far achieved have been reported to be encouraging. There is every likelihood that this interestting uranium occurrence may, in near future, turn out to be an economic deposit.

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ONCOLITES FROM THE ROHTAS LIMESTONE, SON VALLEY AREA, MIRZAPUR DISTRICT, U.P.

ONCOLITES are defined as unattached stromatolites with encapsulating laminae formed by the activity of blue-green algae (Walter[6], 1976). On a recent visit to Kanaura, on Patwadh–Jhiria Forest Road, Dala area, Mirzapur District, U.P., the oncolites are recorded for the Rohtas limestone. This is the first record of oncolites from the Semri Group (Semri Series of Auden[1] 1933) of Son Valley area, Mirzapur District, U.P. (Table I). The only other reference of oncolites in the Vindhyan Supergroup is by Kumar[4] (1977) from the Tirohan Limestone of Chitrakut area, Satna District, M.P., in which they occur in association with phosphorite and columnar stromatolites *Colonella columnaris* and *Baicalia baicalica*.

The oncolites are circular to elliptical in outline with concentric laminae (Fig. 1). The diameter ranges from 1 to 4 cm. These are seen on the upper surface of the bedding plane. The oncolite bearing limestone

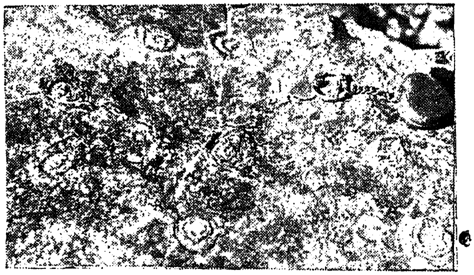

FIG. 1. Oncolites in the Rohtas limestone, Son Valley area, Mirzapur District, U.P.

shows flaser bedding and parallel laminations. The flaser bedding is typical of tidal flat environment of deposition (Reineck and Singh[5], 1973). The of oncolites indicates increased periodic tu usually corresponding to decreased depth, *i.e.*, tidal zone (Gebelein[2], 1976). Kumar[3] (19 recorded poorly developed columnar stromatol the Rohtas limestone of Newari area, Mirzapur U.P. On the basis of the occurrence of onc poorly developed stromatolites it can be that the algal life was present during the dep Rohtas Formation. Although the Rohtas comprises of about 100 meters thick sequence nate rocks, there is a general absence of well- columnar and stratified stromatolites. Howe underlying carbonate horizons show luxuriant of columnar stromatolites *Conophyton*, *Colon* *Kussiella* (Table I). Thus, the absence of loped stromatolites in the Rohtas Formation ascribed to environmental factors.

TABLE I

	Kaimur Sandstone	Sandstones and shales	
SEMRI GROUP	Rohtas Formation	Limestones and shales	Oncolites and poor developed strom
	Kheinjua Formation	Glauconitic Sandstones Fawn Limestone Olive Shales	*Conophyton gargan* *Colonella columnar* *Colonella clappii*
	Porcellenite Formation	Porcellenites	
	Basal Formation	Kajrahat Limestone	*Colonella kajrah* *Conophyton vindhy* *Collenia symmetric* *Kussiella kussiensis* *Kussiella dalaensis*
		Basal Conglomerate	*Kussiella kussiensis*
	Unconformity		
	Bijawar Formation	Slates and phyllites	

The author is grateful to Shri V. K. Sharm help during the fieldwork. Financial help f State Council of Science and Technology, Luc thankfully acknowledged.

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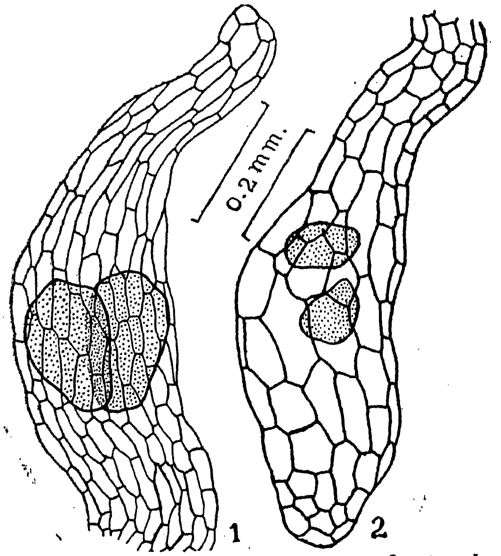
1. Auden, J. B., *Mem. Geol. Surv. India*, 193
2. Cebelein, C. D., *Stromatolites*, Elsevier Publishing Company, 1976, p. 381.
3. Kumar, S., *Jour. Palaeo. Soc. India*, 197
4. —, *Curr. Sci.*, 1977, **46** (10), 341.
5. Reineck, H. E. and Singh, I. B., *De* *Sedimentary Environment*, Springer and 1973.
6. Walter, M. R., *Stromatolites*, Elsevier Publishing Company, 1976, p. 687.

OBSERVATIONS ON THE OCCURRENCE OF POLYEMBRYONY IN TWO SPECIES OF ORCHIDS

POLYEMBRYONY has been observed in some of the Gymnosperms and in some species of Angiosperms. The occurrence of this phenomenon in few species of orchids has been reported earlier[1–5]. While studying the frequency of distribution of polyembryony among orchids, two more species, *viz.*, *Spathoglottis plicata* (Bl.) Bijd. and *Geodorum densiflorum* Schlecht. were found to exhibit this phenomenon under natural conditions. The important features observed in the polyembryonic seeds of the above two species are recorded in this communication.

In both the species studied, the polyembryonic condition is found to be of two embryos only, and these two embryos in a seed are generally lying almost parallel to each other. Further, the twin embryos when compared with that of the normal ones, are found to be remarkably smaller. In the case of *Spathoglottis plicata* (Fig. 1) this difference in size is about 93 × 31 μ and in the other species, *Geodorum densiflorum* (Fig. 2) it is measured to be about 31 × 15 μ. In addition to this, it is also noted that, of the two embryos, one is always slightly smaller than its sister-embryo. However no major difference between the polyembryonic and normal seeds has been noticed either in the shape of the embryos or in the size and shape of the seeds.



FIGS. 1–2. Camera lucida sketches of polyembryonic seeds. Fig. 1. *Spathoglottis plicata* (Bl.) Bijd. Fig. 2. *Geodorum densiflorum* Schlecht.

Polyembryony among Orchidaceae is mainly due either to (1) cleavage of the zygote or proembryo or (2) apomixis. The first type was reported by Swamy[2] in *Cymbidium bicolor* where the resulting seeds were with two embryos each, one among them being slightly smaller. Another form of cleavage polyembryony with some variations was reported in *Eulophia epidendraea* in which case the seeds formed were wth multiple embryos.[3] Apomictic type of polyembryony[5] was recorded in *Zeuxine sulcata* where the number of embryos produced in a seed varied from one to several. In *Gastrodia elata* two embryo sacs in the same ovule often develop to maturity[4]. However, in the present studies, from the number and size of the embryos, it appears that this phenomenon in these two species is due to cleavage, and especially of *Cymbidium type.* The frequency of polyembryony in these two species is found to be as high as about 5%, which is comparatively higher than that reported earlier[2].

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June 20, 1977.

1. Maheshwari, P., *An Introduction to the Embryology of Angiosperms*, London, 1950.
2. Swamy, B. G. L., *Proc. Indian Acad. Sci.*, 1942, **15 A**, 194.
3. —, *Proc. Nat. Inst. Sci. India*, 1943, **9**, 59.
4. —, *Curr. Sci.*, 1943, **12**, 13.
5. —, *New Phytol.*, 1946, **45**, 132.

A SIMPLE TEST FOR ASSESSING COMPARATIVE RESISTANCE OF VEGETABLE FIBRES TO MICROBIAL DAMAGE

To avoid unnecessary economic loss the Vegetable fibres must be graded according to their susceptibility to microbial damage. The present study describes a simple but reliable method of assessing the comparative durability of the fibres against microorganisms. The method proved successful in this respect with ramie fibres having different gum contents.[1]

White jute (*Corchorus capsularis*), tossa jute (*Corchorus olitorius*), mesta (*Hibiscus cannabinus*) and roselle (*Hibiscus sabdariffa*) were obtained from Jute Agricultural Research Institute, Barrackpore, West Bengal; sisal (*Agave sisalana*) from Sisal Research Station, Bamra, Orissa; sunnhemp (*Crotalaria juncea*) from Sunnhemp Research Station, Pratapgarh, U.P.; *banana* (*Musa sapientum*) from Khadi and Village Industries Commission, Bombay and Manila (*Musa textilis*) from a private source. The fibres were first sorted to eliminate the defective and unclean fibres. The selected fibres were then tested for their initial tenacity. The fibres were then sampled and bundles 8″ in length and 15 g in weight were incubatesd at 30° C with 30° g sterile distilled water in sterile petri dishes under covered condition for different periods of time. Loss of moisture in the petri dishes during incubation was made up aseptically with sterile distilled water once a week. At definite intervals of time, representative samples were taken out, washed first with water and then with rectified spirit and finally dried in air. The fibre strength was then tested in J.T.R.L. bundle strength tester[2] and the extent of retention of strength was taken as an index of extent of resistance to microbial damage.

Results are presented in Table 1 from which the fibres may be graded in the order of decreasing resistance to microbial damage as Manila > sisal > mesta > sunnhemp > *banana* > tossa jute > white lute.

TABLE I

Comparative resistance of different fibres to microbial damage

Fibre	% retention of tensile strength after incubation for			
	15 days	1 month	2 months	3 months
White jute	64·9	60·4	36·0	7·2
Tossa jute	68·5	47·6	25·0	14·9
Banana	78·7	37·0	34·3	15·0
Sunnhemp	43·4	32·7	27·3	20·6
Mesta	73·2	51·4	44·6	34·5
Sisal	80·5	66·7	70·2*	52·5
Manila	100	99·6	86·9*	91·3

N.B.—Results were calculated on the average of 3 replications.

* These anomalies may be due to minor sample variations.

In the method described, the clean and undamaged fibres were sampled under identical conditions and during the period of sampling all the fibres were under the same microbial exposure. As such, there was no significant difference in the initial microbial population of the fibres. Obviously, incubation in moistened state with sterile distilled water under covered condition at 30° C allowed the predominant growth and activity of the microorganisms (mainly fungi and bacteria) in their normal association to degrade the fibre cellulose proportionately as the susceptibility to damage.

Reports so far published[3,4] indicate that the resistance to damage of a fibre is mainly related to its physical properties, *viz.*, crystallinity, chain length of cellulose and mode of association of fibre constituents and its chemical composition, *viz.*, lignin/hemicellulose ratio and presence of some minor constituents. The findings of the present study are more or less concordant with the reasoning as evidenced from available data on different fibres[5-7]. In general higher the crystallinity, chain length and lignin/hemicellulose ratio values, less is the microbial damage.

The methods of testing resistance of fibres to damage includes soil burial test and mixed culture inoculation method[8]. The former is the universal method of testing rot resistance and is most suitable for testing fibre goods treated with antiseptics. The mixed culture test is no doubt every sound but is a bit cdmbersome as the propagation and maintenance of five different cellulolytic fungi are involved in it. Along with the above two methods, the method mentioned here may be used as a very simple and inexpensive technique for comparing the resistance of different cellulosic fibres and fibre goods to microbial damage without any serious drawback.

The authors are indebted to Dr. A. C. Chakravarty, Director, Dr. S. B. Bandyopadhyay, former Director and Dr. P. C. Das Gupta, Head of the Division of Chemistry, for encouragement and facilities. Sincere thanks are also due to Shri N. Geo Paul for his kind co-operation.

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1. Bhattacharyya, S. K. and Paul, N. B., *Curr. Sci.*, 1976, **45**, 417.
2. BandyoPadhyay, S. B., *Jute Bulletin*, 1965, **27**, 278.
3. Bose, R. G., *Sci. and Cult.*, 1952, **17**, 435.
4. Desai, A. J. and Pandey, S. N., *J. Sci. Ind. Res.*, 1971, **30**, 598.
5. Meredith, R., *Text. Prog.*, 1975, **7** (4), 1,
6. Das Gupta, P. C. and Mazumdar, A. K., *Institute of Jute Technology, Annual Number*, 1971, p. 19.
7. Mazumdar, A. K., *J. Sci. Ind. Res.*, 1955, **14B**, 542.
8. Indian Standard Institution, New Delhi, *Specifications IS* 1623, 1960.

A NEW DISEASE OF *SOLANUM KHASIANUM* C. B. CLARKE emend SEN GUPTA

DURING the survey on diseases of solanaceous plants, the authors observed an interesting disease of *Solanum khasianum* caused by *Colletotrichum capsici* (Syd.) Butl. and Bisb. which is not reported[1-2] earlier from India.

All the aerial parts are susceptible to the disease, which generally appeared at the collar region as dark-brown lesion which deepened and gradually extended to the upper aerial branches. The tender aerial parts showed 'epinasty' followed by premature defoliation. In severely affected plants, yellowing of leaves was conspicuous and the plants ultimately died prematurely. At the advanced stage, black bodies made their appearance which were identified as *Colletotrichum capsici* (Syd.) Butl. and Bisb.

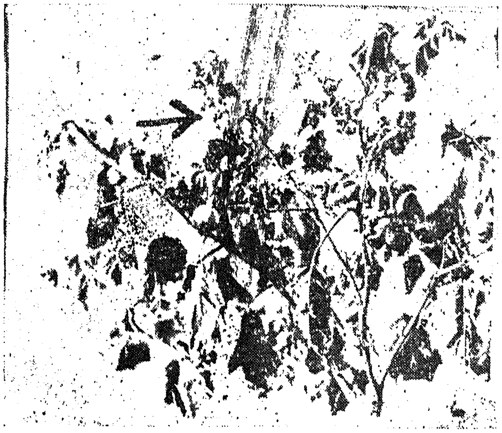

FIG. 1. A branch of *Solanum khasianum* showing the symptoms of the disease caused by *Colletotrichum capsici*.

Pathogenicity of the organism was established by spraying spore suspension in sterilized water as well as mass inoculation after a scalpel injury. The inoculated plants were protected by covering with sterile polythene bags. Sufficient moisture was maintained by placing wet cotton pad over the inoculum. Characteristic symptoms developed after 5–7 days. Isolations from the induced disease tissue yielded the same organism. The disease was more prevalent during October–November months during which high humidity and favourable temperature prevailed.

Thanks are due to Prof. U. B. S. Swami, Professor and Head, Department of Botany, Kakatiya University, for providing facilities.

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---

1. Butler, G. J. and Bisby, G. R., *The Fungi of India*, Revised by R. S. Vasudeva, I.C.A.R., New Delhi, 1960.
2. Mukerji, K. G. and Juneja, R. C., *The Fungi of India*, Emkay Publications, New Delhi, 1974.

AMYLASE ACTIVITY IN A BLUE-GREEN ALGA

ELECTRON microscopic studies in blue-green algae have revealed the presence of interlamellar granules such as α-granules[1,2]. These were further isolated into a pure state from the blue-green alga *Nostoc muscorum*[2]. Chemical analysis of the α-granules suggested these to contain highly branched polyglucosides. Degradation of the isolated α-granules by α-amylase was also achieved *in vitro*[3]. The α- and β-amylases and their isoenzymes of the blue-green alga *Anabaena ambigua* were studied by Wahal *et al.*[4] Since a comparative account on the activity of α-amylase in the vegetative cells and spores of blue-green algae is lacking, the present work was undertaken.

The filamentous, heterocystous and sporulating blue-green alga *Anabaena* sp. was employed during the present study. The alga was grown in the nitrogen-free medium of Allen and Arnon[5]. The cultures were maintained at 30 ± 1° C and illuminated with daylight fluorescent tubes. Log-phase cultures and spores were used for obtaining cell-free preparations. Two methods were tried. In the first method, the enzyme extracts were prepared by directly grindng the cells in buffer in a pre-chilled glass mortar. Significant activity of the enzyme could not be detected from these extracts. So the second method was adopted further in which the cell-free preparations were obtained from protoplasts. Log-phase cultures of the alga were concentrated by centrifugation, washed with sterilized water, followed by washings with 0·03 M sodium phosphate buffer (pH 6·8). The cells were resuspended in 10 ml solution consisting of phosphate buffer, 0·5 M mannitol and 10 mg lysozyme (BDH, London, 3 X crystallized). The suspension was incubated in a water bath at 37 ± 1° C for 4 hours to obtain protoplasts[6,7]. Microscopic examination revealed the formation of protoplasts. Initial attempts to wash the protoplasts revealed the loss of amylase activity in the supernatant of the centrifuged protoplast suspension. Hence no further attempts were made to free the protoplast suspension from the added lysozyme. The suspension was then macerated in a glass mortar at 4° C for 10 min with 0·5 g of acid-washed sand. The leaching out of the biliprotein pigments indicated the disruption of the protoplasts. It was then centrifuged at 3,000 rpm for 10 min to remove cell debris. The process of maceration was repeated thrice followed by intermitent washings with cold buffer-manitol solution. The cell-free extract thus pooled to a final volume of 25·0 ml was utilized as crude extract for the assay of α-amylase.

Amylase activity was measured by incubating 1 ml of the cell-free extract with 2 ml of 2% soluble starch solution (dissolved in PO_4-mannitol buffer) and 2 ml of buffer solution, pH 6·8, for 1 hour at 37 ± 1° C.

Necessary precautions were taken to prevent bacterial growth in the reaction mixture under incubation. The increase in reducing power was determined by the addition of 3, 5-dinitro salicylic acid reagent[8]. The protein content of the cell-free preparation was determined by the method of Lowry *et al*[9]. While calculating the protein content of the extract the amount of lysozyme added was subtracted from the total protein content. The experiments were performed in duplicate and each experiment was carried at least 2–3 times.

The presence of amylase activity in the supernatant fractions of centrifuged protoplast suspension indicates the release of the enzyme from the protoplasts. This is in agreement with the observations of Heinen and Lauwers[10] who studied the amylases released by the protoplasts of *Bacillus caldolyticus*. The results are summarized in Table I. It is apparent that the actively growing cell suspension showed comparatively less activity of the enzyme than that of vegetative cells. It is now established that the accumulation of glycogen takes place in photosynthesizing conditions and the degradation in photosynthetically inhibited cultures or in dark[11]. As the log-phase cultures employed during the present study were directly taken from light, it may be that a basal level of utilization of glycogen persists even in light. The isolated spores of *Anabaena cylindrica* fixed CO_2 in light at considerably lower rate than vegetative cells[12]. Hence it is probable that the spores have to depend largely on the glycogen reserves. The high amylase activity observed in the spores during the present study supports the above conclusion.

TABLE I

Amylase activity in Anabaena *sp.*

Phase of culture	Amylase activity mg maltose/h/mg protein	
	Range	Average
Vegetative cells	1·368–2·933	2·150
Spores	6·487–8·000	7·243

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1. Giesey, R. M., *Amer. J. Bot.*, 1964, **51**, 388.
2. Chao, L. and Bowen, C. C., *J. Bact.*, 1971, **105**, 331.
3. — and —, In *Taxonomy and Biology of Blue-green Algae*, T. V. Desikachary (ed.), 1972, p. 13.
4. Wahal, C. K., Bhattacharya, N. C. and Talpasayi, E. R. S., *Biochem. Physiol. Pflanzen*, 1974, **165**, 351.
5. Allen, M. B. and Arnon, D. I., *Plant Physiol.*, 1955, **30**, 366.
6. Biggins, J., *Ibid.*, 1967, **42**, 1442.
7. Bhattacharya, N. C. and Talpasayi, E. R. S., *Arch. Mikrobiol.*, 1973, **90**, 157.
8. Bernfeld, P., in *Methods in Enzymology*, S. P. Colowick and N. P. Kaplan (eds.), 1955, **1**, 149.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
10. Heinen, W. and Lauwers, A. M., *Arch. Mikrobiol.*, 1975, **106**, 201.
11. Lehman, M. and Wober, G., *Ibid.*, 1976, **111**, 93.
12. Fay, P., *J. Exptl. Bot.*, 1969, **20**, 100.

VAUCHERIA BILATERALIS JAO.— A NEW RECORD FROM INDIA

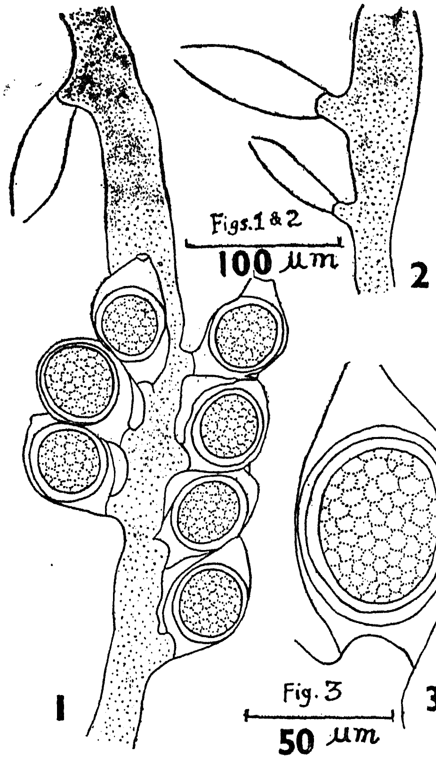
THE genus *Vaucheria* is represented in India by nine species[1], although the genus contains 54 species in all[2].

In the course of studies on the algal flora and its periodicity in the river Gomati (U.P.), a species of *Vaucheria* hitherto unrecorded from India was found. It is proposed to record the taxon in the Indian flora and to briefly describe the Indian plant.

The alga was found growing in January 1976, floating in a back-water portion of the river near the bank in Lucknow (U.P.). It formed a bright green woolly mat-like patch on the surface of the water having scanty flow.

The main siphonaceous filaments of the alga measure 20–40 (–50) µm in diameter. In this respect the alga is slightly narrower than the type species which is (32–) 40–48 µm across. Branching is lateral and monopodial and the distal branches are slightly narrower. The vegetative morphology is quite similar to other species of the genus.

Zoosporangia were not found in the material and no other mode of asexual reproduction was observed. Sexual reproduction was abundant in the material at the time of collection. Oogonia were subsessile or possessed a short stalk and were formed in series on two opposite lateral sides of the main axis. The number of oogonia on each side ranged from one to six (Fig. 1). The fertilised oogonia possessed a prominent pore-like opening at the distal end. The oogonia measured 40–45 µm in breadth and 60–88 µm in length. Plants are monoecious. Antheridia may occur on separate branches (Fig. 2) or on the same branch as oogonia (Fig. 1). Antheridia are narrower, slightly inflated, cylindrical sub-tubular structures formed singly on one side only or one on either side of the fertile axis above the series of oogonia. They possess a very short stalk, measure 17–32·5 µm in breadth and 50–92·5 µm in length and possess a terminal opening. Mostly, empty antheridia were found in the material. The oospores are globose to subglobose in structure with a thick, three-layered outer membrane. The layers are smooth and the middle layer is yellow brown in colour. The globose oospores measure 45–48 µm in diameter while the sub-globose oospores measure 45–49 µm in breadth and 56–64 µm in length.



FIGS. 1–3. *Vaucheria bilateralis* Jao. Fig. 1. Bilaterally arranged oogonia in series containing globose oospores and an empty antheridium above. Fig. 2. A branch with two empty antheridia. Fig. 3. An oogonium containing a subglobose oospore.

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---

1. Venkatraman, G. S., *Vaucheriaceae,* Indian Council of Agri. Res., New Delhi, 1961.
2. Jao, C. C., "Studies on Fresh water Algae of China II, Vaucheriaceae from Szechwan," *Sinensia,* 1936, 7, 740 (original not seen).

RAPID BIOCHEMICAL TEST TO DETECT ROOT (WILT) DISEASE OF COCONUT

THE root (wilt) disease is the most serious malady of coconut. Its etiology is still unknown. The disease can be identified on the basis of morphological symptoms[1] and serological test[2]. In the present study an attempt was made to develop a simple and quick biochemical test using EDTA as an extractant of biologically active organic substances/pigments present in the diseased palms.

Two hundred and forty adult palms and sixty seedlings growing in diseased zone and 105 adults and 45 seedlings growing in healthy zone, all belonging to West Coast Tall variety in three soil types, *viz.,* sandy loam/red sandy loam, laterite and coastal sandy, were selected for this study. Equal number of palms/seedlings were selected in each category. 85 palms including seedlings, growing in diseased and healthy zones, on which serological test was performed, were also examined in this study.

0·3 M solution of ethylene diamine tetra-acetic acid-disodium salt was prepared and its pH was adjusted to 6·8–7·0 by adding NaOH solution. Two leaflets each from both sides of the rachis of spindle and root-tips from five actively growing roots were collected and kept in an ice box. 0·5 cm diameter leaf discs were cut out from top, middle and basal portions of external moisture-free leaflets with the help of a cork borer. Those discs (1 g) were transferred to a petridish containing 10 ml EDTA solution. Each disc was divided into four pieces by giving parallel 3 cuts with the help of scissors and forceps. The cut discs were steeped in the solution for 5–7 minutes with four occasional shakings. After completion of steeping, the EDTA extract was decanted and poured into a test tube. In another set, tender root tips of about 6 cm length were washed properly with water, blotted carefully and cut into small pieces to a length of about 0·5 cm. These pieces were mixed thoroughy, weighed to 1 g, transferred to EDTA solution, cut into fine pieces by a clean blade, steeped and thereafter decanted the extract as mentioned in case of spindle. The transmittance of spindle and root extracts was read using blue filter (400–450 mµ wavelength) in the Klett Summerson colorimeter. The colour of EDTA extracts was identified with the help of the 'Dictionary of Colour'[3]. This test was performed during rainy/wet and summer/dry seasons and in each season the tissues were tested

TABLE I

Colour and transmittance values of spindle (S) and root (R) extracts and R/S transmittance ratios in healthy and root (wilt) diseased coconut palms during different seasons

Season		Healthy	Early/ Latent diseased	Advanced diseased
Summer (Dry)	Spindle	Yellowish brown (antiquigold) >140	Yellowish light brown (cloudy amber) <140	Yellowish verylight brown <100
	Root	Brown <330	Dark brown >440	Dark brown >440
	R/S Transmittance	1·86±0·57	4·19±1·17	5·01±0·99
Rainy (wet)	Spindle	Yellowish brown (antiquigold) >100 >80*	Yellowish light brown (cloudy amber) <100 <80*	Yellowish very light brown <70 <60*
	Root	Brown <300	Dark brown >400	Dark brown >400
	R/S Transmittance	1·90±1·03	4·000±1·03	5·05±0·89

C.D. for transmittance values = 6·6 (Significant at 0·1% level).
* Values recorded within 24 hours after rain.

twice. The average value in each case is presented in Table I.

The spindle and root extract cf diseased palms showed yellowish light brown (cloudy amber[3]) and dark brown colour respectively. On the contrary, in healthy palms the spindle and root extract exhibited yellowish brown (antiquigold[3]) and brown colour respectively. The transmittance values recorded for spindle and root exract of diseased and healthy palms differed significanty. In apparently healthy palms (healthy palms of diseased zone), the spindle-transmittance was equivalent to that of healthy ones but the root-transmittance was found to be in between the values of healthy and early/latent diseased palms. The values recorded for adult palms and seedlings did not differ significantly even with variation in the soil types. But the transmittance values recorded for spindle and root extracts during summer/dry season reduced markedly by the onset of rains/rainy season (Table I).

With the severity of disease, the transmittance values of spindle and root extracts decreased and increased respectively. Exceptions to an extent of 15% were recorded, where the spindle-transmittance values of diseased palms did not differ significantly from that of healthy ones and root-transmittance values of healthy palms from that of diseased ones. Such cases were usually observed in those diseased palms, where there were no traces of spindle-rot but had severe attack of insects specially *Myllocerus curvicornis* F. However when transmittance ratio of root/spindle was worked out, it was found to be 1·86 ± 0·57, 4·19 ± 1·17 and 5·01 ± 0·99 for healthy, early diseased and disease advanced palms respectively. The corresponding ratios for rainy season differed insignficantly. The root/spindle ratio was therefore taken as another important crtierion for detecting the different degrees of disease. It is evident from these results that there is some imbalance in the synthesis/distribution of organic substance/pigment in spindle and roots of diseased palms. The chemical nature of this pigment is not yet known and needs further investigation.

The values fixed for different intensity of disease by this test coincided to an extent of 85% and more with the visual symptoms and serological test. Moreover, this biochemical test is simple, reliable and takes hardly 10 minutes time to distinguish between healthy and diseased palms of different degrees.

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---

1. Radha, K. and Lal, S. B., *Indian J. of agric. Sci.*, 1972, **42**, 410.
2. Solomon, J. J., Sasikala, M. and Shanta, P., *Abstract, International Symposium on Coconut Research and Development*. December 28 to 31, 1976, Kasaragod (India).
3. Maerz, A and Paul, M. R., *Dictionary of Colour*, McGraw-Hill, N.Y., 1950.

CHROMOSOME NUMBER OF *ANTHERAEA FRITHII* MOORE (LEP: SATURNIIDAE)

Antheraea frithii Mr., known to produce tasar silk (Jolly *et al.*[1], 1968) is commercially unexploited. It is endemic in eastern hilly tracts of India (Meghalaya, Manipur, etc.) at altitudes 900–1,200 msl and feeds primarily on *Terminalia*. Detailed morphology of this species has been studied by Jolly *et al.*[4] (1974). However, the insect remained untapped so far as its cytological aspects are concerned. The present communication deals with the chromosome number of *A. frithii* Mr. collected from Manipur and the cytological behaviour observed in the course of interspecific hybridisation studies.

Testes from live pupae were dissected two weeks prior to emergence, fixed in acetic-alcohol (1:3) for 4 h and then preserved in 70% alcohol. Temporary acetocarmine squashes were prepared following the staining technique of Jolly *et al.* (1970). The chromosome counts of the metaphase spermatocytes were carried out at 16 × 100^x magnification, the camera lucida diagrams drawn and microphotographed (Fig. 1).

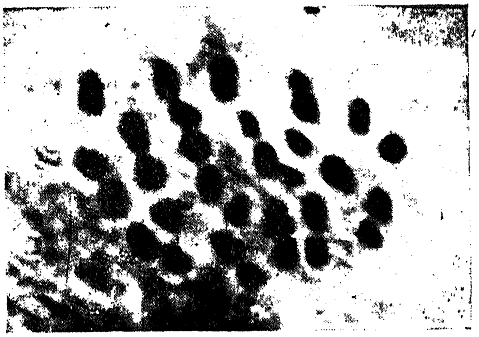

FIG. 1. Polar view of metaphase I showing haploid chromosome number of *A. frithii* Mr. ($n = 31$).

The chromosome number established for this species is $n = 31$. The chromosomes were oval and variable in size, as revealed by Fig. 1.

Studies on interspecific hybridisation between *A. frithii* ($n = 31$) and *A. mylitta* ($n = 31$; Sinha and Jolly[5], 1967) showed that though the F_1 progenies were healthy, the adults were sterile (Jolly *et al.*[3], 1971). The cytological investigation of the F_1 individuals indicated lack of synapsis in most of the cases resulting in 62 (31 + 31) chromosomes during I meiotic metaphase. Some of the cells however exhibited pairing tendency of the chromosomes. These observations suggest a possible close phylogenetic relationship between *A. frithii* Mr. and *A. mylitta* D.

It needs mention here that *A. frithii* is the seventh *Antheraea* species whose chromosome number has been elucidated.

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---

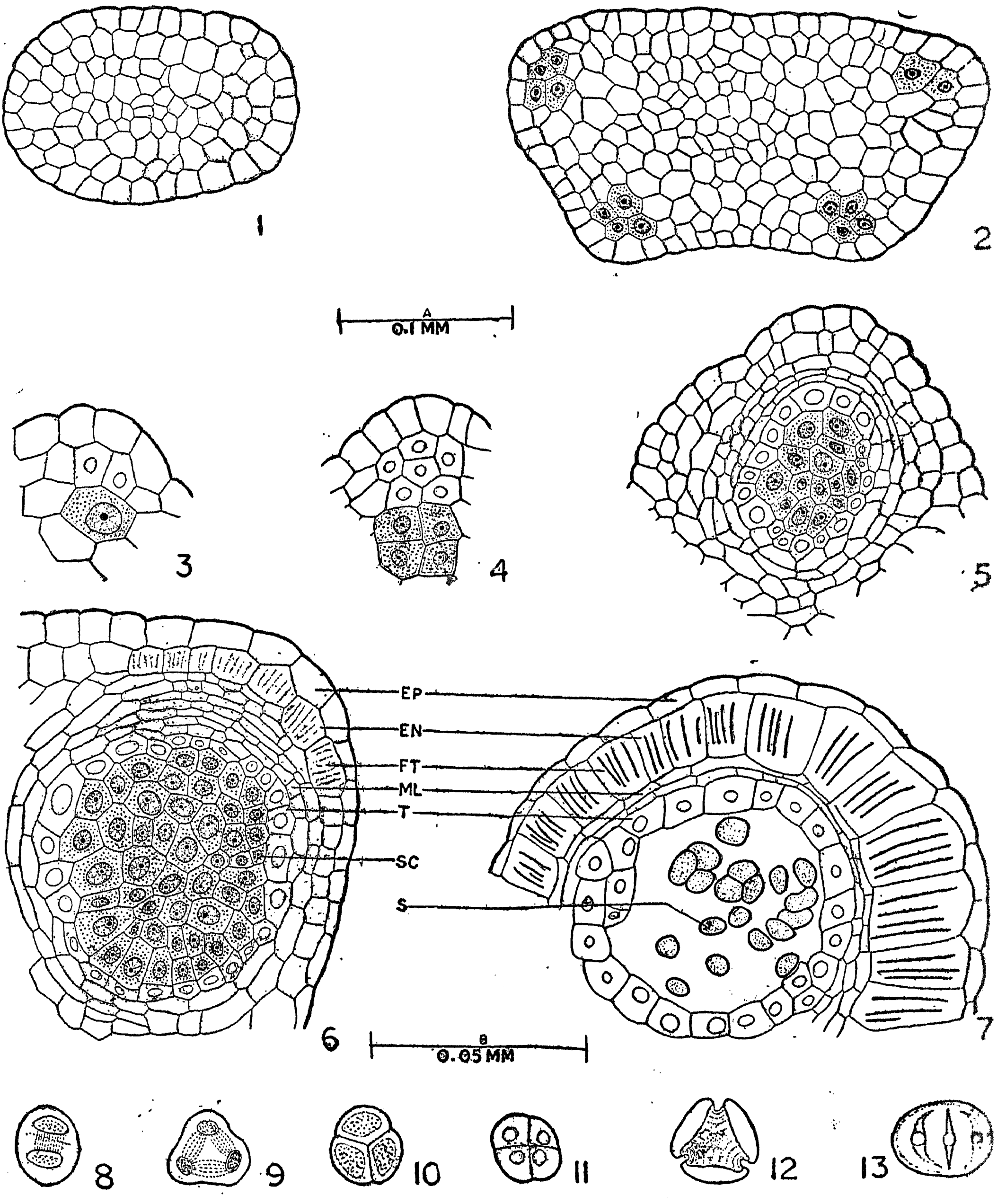
1. Jolly, M. S., Prasad, S. and Chaturvedi, S. N., *Ind. J. Seri.*, 1968, 7, 56.
2. —, Sen, S. K. and Sinha, S. S., *Ind. J. Ent.*, 1970, **32**, 91.
3. —, Narasimhanna, M. N., Sen, S. K. and Bardaiyar, V. N., *Ind. J. Seri.*, 1971, **10**, 42.
4. —, — and Sen, S. K., *Ibid.*, 1974, **13**, 23.
5. Sinha, S. S. and Jolly, M. S., *Curr. Sci.*, 1967, **36**, 359.

MICROSPOROGENESIS IN *LITCHI CHINENSIS* L.

THE embryology of Sapindaceae has received very little attention by earlier workers[1]. Very little is known about the microsporogenesis in *Litchi chinensis*[2]. Hence the present work was undertaken. The floral buds of this species in various stages of development were collected from Horticultural Research Institute, Saharanpur and fixed in Carnoy's fluid. Customary methods of dehydration infiltration and embedding were followed. Sections were cut at 6–8 microns and stained with hematoxylin.

In very young stages of development, the anther appears somewhat oval in cross-section (Fig. 1), having a homogeneous mass of meristematic cells. Later on it becomes somewhat angular and differentiates into four anther lobes (Fig. 2). One, two or sometimes more archesporial cells are differentiated in each anther lobe (Fig. 2). These cells are more conspicuous with dense cytoplasm and prominent nuclei. The archesporial cells elongate radially and divide periclinally to form an outer primary parietal layer and an inner mass of large primary sporogenous cells (Figs. 3, 4). The primary parietal layer further divides periclinally and anticlinally to form the wall layers (Figs. 5–7). The outermost layer of the anther is epidermis. A layer, next to the epidermis, is the endothecium (Figs. 6, 7) which, in fully mature anther, develops fibrous thickenings from its inner tangential walls (Figs. 6, 7). The middle layers vary from 2 to 3 or more cells in thickness (Figs. 6, 7). They gradually become flattened and ultimately degenerate even earlier than the cells of the tapetum. The tapetum is glandular in nature and its cells are full of granulated cytoplasm. Finally the tapetal cells elongate radially and then degenerate.

The primary sporogenous cells divide and redivide to form microspore-mother-cells which are compactly arranged (Figs. 4, 6). The microspore-mother-cell divides meiotically to form first a diad (Fig. 8) and then a tetrad (Figs. 9–11). The microspore tetrads



FIGS. 1–13. Microsporogenesis in *Litchi chinensis* L. Fig. 1. Cross-section of anther in very ea stage of development. Fig. 2. Cross-section of anther showing the archesporial cell differentiation. Figs. 3 4. Cross-sections of anther lobes showing stages in microsporogenesis. Figs. 5–7. Stages in the developmen wall layers in an anther lobe in cross-sections. Fig. 8. A diad formation. Fig. 9. A tetrad format Fig. 10. A tetrahedral tetrad. Fig. 11. An isobilateral tetrad. Figs. 12, 13. A single microspor polar and equatorial view respectively.

EN—Endothecium; EP—Epidermis; FT—Fibrous thickenings; ML—Middle layer; S—Microsp SC—Sporogenous cell; T—Tapetum. Scale A for Figs. 1, 2, 5–7. Scale B for Figs. 3, 4, 8–13.

are usually arranged in a tetrahedral manner (Fig. 10) and rarely they are isobilateral (Fig. 11). Later on the microspores separate from each other in a tetrad. A single celled microspore is a triangular or a rounded structure (Figs. 12, 13).

The authors are thankful to the Director, Horticultural Research Institute, Saharanpur for their help in procuring the material used in this study and to Dr. V. Singh for his valuable suggestions and laboratory facilities.

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---

1. Davis, G. L., *Systematic Embryology of Angiosperms*, John Wiley and Sons, Inc., New York, 1966.
2. Banerji, I. and Chaudhuri, K. L., *Proc. Indian Acad. Sci.*, 1944, **19 B**, 19.

PHYSIOLOGICAL CHARACTERIZATION OF A SPICE (*CORIANDRUM SATIVUM*) AND A CONDIMENT (*TRIGONELLA FOENUM GRAECUM*) DURING VEGETATIVE AND REPRODUCTIVE STAGES

THE physiology of spices and condiments has not been studied in detail. The astringency and flavour which they impart may be due to phenolics, alkaloids, terpenoids and tannins. Tannins are remarkable for their astringent or 'puckery taste'[1]. Vegetable tannins are polyhydric phenols. The present study was undertaken to have an idea as to the nature of the phenolic substances which may provide some physiological markers for a spice and a condiment.

Seeds of coriander and trigonella were sown in the garden plots. Qualitative and quantitative estimation of phenolic acids was made by using two-dimensional paper chromatography with benzene : acetic acid : water (60 : 20 : 30) in the first direction and sodium formate : formic acid : water (10 : 1 : 200) in the second. The dried sheets were observed under ultraviolet light and were sprayed with diazotized *p*-nitroaniline or diazotized sulphanilic acid. The extraction and estimation of phenolic acids was made according to the method of Bate-Smith[2].

The total phenol content of coriander was more than that of trigonella during the vegetative and reproductive phases. Trigonella was characterized by more number of phenolic acids when compared to coriander both in vegetative and reproductive phases. Rutin, caffeic acid, cis and trans *p*-coumaric acids and trans-ferulic acids were not present in coriander during vegetative stage. Cis and trans forms of *p*-coumaric acids were observed at the reproductive phase apparently indicating that these two acids may contribute to the spicy nature of coriander which can be substantiated by the highest content of these acids in the dry seeds of coriander. The formation of caffeic acid and chlorogenic acid shows the involvement of shikimic acid pathway in phenolic biosynthesis[3]. The presence of protocatechuic acid is another evidence for the involvement of shikimic acid pool[4]. Protocatechuic acid, chlorogenic acid, *p*-coumaric acids which are the intermediates of shikimic acid are less in trigonella compared to coriander indieeting that the spice coriander is more fibrous (because of more lignin *via* shikimic acid pathway) than the condiment trigonella. Thus, phenolics provide physiological

TABLE I

Phenolic acids in coriander (spice) and trigonella (condiment)

Phenolic compound (µg/gm fr. wt.)	Coriander			Trigonella		
	Vegetative stage	Reproductive stage	Dry seed	Vegetative stage	Reproductive stage	Dry seed
Protocatechuic acid	179	167	760	36	60	..
Chlorogenic acid	305	320	..	80	90	..
p-hydroxybenzoic acid	252	333	960	227	260	480
Vanillic acid	221	347	960	302	360	750
Rutin	..	..	..	111	100	260
Rutin derivatives	..	..	..	..	110	..
Caffeic acid	..	..	..	27	60	..
Cis *p*-coumaric acid	..	273	1440	53	105	260
Trans *p*-coumaric acid	..	173	1120	80	90	..
Trans ferulic acid	..	..	..	111	80	..
Cis ferulic acid	..	467	1360	..	320	..
Cinnamic acid	..	40	..	..	..	..
Total phenols	45	51	..	30	15	..

Note.—The total phenol content is expressed as µg/gm fresh weight.

markers for differentiating a spice from a condiment. Jindal and Singh[5] studied the phenolic content in male and female *Carica papaya*, which served as possible physiological marker for sex identification of vegetative seedlings.

Thus in a final assessment of the data, it is clear that the physiology of spice is different at least in some respects from that of a condiment and the terminology of spice and condiment holds good basing on physiological criteria.

The authors thank Prof. V.S. Rama Das for providing facilities and constant encouragement.

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---

1. Joslyn, M. A. and Goldstein, J. L., *Advan. Food Res.*, 1964 *a*, **13**, 179.
2. Bate-Smith, E. C., *Biochem. Jour.*, 1954, **58**, 123.
3. Neish, A. C., *Ann. Rev. Plant Physiol.*, 1960, **11**, 55.
4. Gross, S. R., *Jour. Biol. Chem.*, 1958, **233**, 447.
5. Jindal, K. K. and Singh, R. N., *Physiologia Plantarum*, 1975, **33**, 104.

INFLUENCE OF POTASH NUTRIMENT ON PHENOL AND SOLUBLE CARBOHYDRATES IN CHILLI LEAVES

POTASSIUM fertilization has been reported to reduce the intensity of paddy blight caused by *Xanthomonas oryzae* (Ono[8], 1957; Ranga Reddy and Sridhar[11], 1976), High levels of potash decreased total phenolics and sugar levels and *X. oryzae* infected leaves accumulated more phenols and low reducing and non-reducing sugars (Ranga Reddy and Sridhar[11], 1976). In the present study the effect of potash application against *X. vesicatoria* in chillies has been studied.

Materials and Methods

Chilli variety, K 1, was transplanted in pots and applied with six levels of potassium, *viz.*, 0 K, 30 K, 60 K, 90 K, 120 K kg/ha. Inoculation was done with the bacterial suspension (*ca.* 10^7 cells/ml) of *X. vesicatoria*, when the plants were 35 days old and the pots were kept under humid condition for the disease development. Each treatment was replicated four times and suitable controls were also maintained. After 5 days, when the plants developed the disease, plant samples were collected from all the treatments and extracted with 80% boiling ethanol. The ethanol extract was used for further estimations of total phenols, (by Bray and Thorpe[2], 1954), reducing sugars (by Nelson[7], 1944) and non-reducing sugars (by Inman[4], 1962).

Results and Discussion

Increased levels of potash application has decreased the total phenolics' concentration (Table I). Potassium-deficient plants contained large amounts of phenols (Mulder[5], 1949). It is presumably due to high amounts of soluble sugars in the plants for the synthesis of phenols. Bacterial inoculation accumulated more total phenolics (Table I). The recent report of Purushothaman[10] (1975) also indicates that the rice plants infected with *X. oryzae* accumulated more phenolics. This increase in phenolic concentration might arise from the release of phenols from their glucosides by the B-glucosidase of either host or pathogen (Pridham[9], 1965). Nayudu and Walker[6], (1961) reported that high levels of N, P and K suppressed the chilli bacterial leaf spot by disturbing the osmotic pressure. Potassium may also lead to disturb nitrogen and carbohydrate metabolism (Hewitt[3], 1963). Higher amounts of soluble carbohydrates (Bird[1], 1954) are reported to favour tissue susceptibility especially to *Xanthomonas* species. In the present case, as the potash level increased, the soluble carbohydrates decreased (Table II).

TABLE I

Changes in total phenols of healthy and X. vesicatoria *infected chilli leaves in Catechol equivalents* (μ*g/g oven dry weight*)

K level in gm/pot	Phenol level	
	Healthy	Infected
Control	325	296
0·30	309	324
0·60	216	245
0·90	193	229
1·20	248	232

TABLE II

Changes in reducing and non-reducing sugars of healthy and X. vesicatoria *infected leaves (glucose equivalents* μ*g/g oven dry weight)*

K level in gm/pot	Reducing sugars		Non-reducing sugars	
	Healthy	Infected	Healthy	Infected
Control	696	683	1650	1440
0·30	671	643	1500	1305
0·60	587	536	940	897
0·90	512	480	680	664
1·20	506	523	590	576

In general, the plants supplied with high potash tend to increase permeability of the protoplasmic membrane (Stocking[13], 1956). In leaves supplied with potassium, phenolic compounds may accumulte around the diseased spots and thereby arrest the multiplication and migration of the bacterium, to control the severity of the disease.

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1. Bird, L. S., *Plant Dis. Reptr.*, 1954, **38**, 653.
2. Bray, H. G. and Thorpe, L. V., *Meth. Biochem Anal.*, 1954, **1**, 27.
3. Hewitt, E. J., *Inorganic Nutrition of Plants*. Academic Press, New York, 1963, **3**, 137.
4. Inman, R. E., *Phytopathology*, 1962, **52**, 1207.
5. Mulder, E. G., " Mineral nutrition in relation to the biochemistry and physiology of potatoes," 1949, **2**, 59.
6. Nayudu, M. V. and Walker, J. C., *Phytopathology*, 1961, **51**, 368.
7. Nelson, N., *J. Biol. Chem.*, 1944, **15**, 375.
8. Ono, K., *Potassium Symp. (Japanese) International Potash Inst., Berne, Switzerland*, 1957, p. 137.
9. Pridham, J. B., *Ann. Rev. Pl. Physiol.*, 1965, **16**, 13.
10. Purushothaman, D., *IL RISO.*, 1975, **25**, 85.
11. Ranga Reddy, P. and Sridhar, R., *Indian Potash J.*, 1976, **1**, 13.
12. Sridhar, R., *Acta Phytopath. Acad. Sci. Hung.*, 1972, **7**, 57.
13. Stocking, C. R., *Encyclopedia of Pl. Physiol. of the Plant Cell*. Springer-Verlag, Berlin., 1956, p. 22.

PLEUROTUS EOUS (BERK) SACC.: A NEW CULTIVATED MUSHROOM

During the survey of edible mushrooms around Mysore, an attractive pink coloured mushroom, similar to *P. flabellatus* (white coloured), was collected during October 1975 from the dead portion of a living tree (*Ficus bengalensis*). The identification was confirmed by Dr. D. N. Peglar of Royal Botanic Gardens, Kiew, as *Pleurotus eous* which was earlier reported in 1848 from hot valleys of Sikkim, India[*].

The cultivation of *P. eous* (Fig. 1, A–C) was successfully carried out on water soaked chopped paddy straw in circular plastic basins (32 cm dia × 13 cm) shallow and deep rectangular wooden trays (60 × 90 × 17·5 cm) at room temperature (21–35° C) and R.H. 65–90%. About 100 g, spawn on paddy straw, 25–30 g of coarsely ground horse gram and 3 kg of pre-soaked paddy straw were mixed and filled into containers to raise the mushroom crop. During the cultivation, it was observed that the deep wooden trays required less watering and provided 1·6–1·8 times more yield compared to the circular basins and shallow wooden trays. The number of pinheads formed in shallow containers were often more compared to the deep trays but the yield recorded from the latter was much higher. This may be attributed to the fact that lower number of pinheads grew to their maximum size. The substitution of oilseed cakes (groundnut/sesame) at different levels, while spawning, did not improve the mushroom growth.

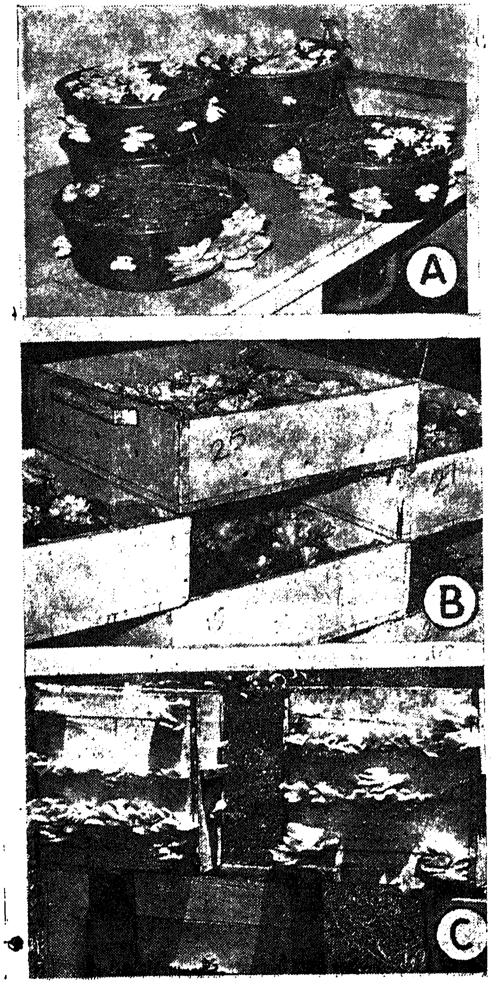

Fig. 1. *Pleurotus eous* growing in circular plastic basins (A), rectangular shallow (B) and deep (C) wooden trays.

Proper ventilation was found necessary for cultivation. The colour of *P. eous* was distinctly pink when young but faded slightly with growth. The colour of mushroom was darker when grown at lower temperatures (22–28° C).

Proximate composition and nutritive constituents of *P. eous* was found to be similar to that of other species of *Pleurotus*[1-3]. The protein content was 33·24% on dry weight basis. The free amino acids were extracted in cold, with 80% ethyl alcohol, purified by passing through Dowex-50 column and separated by high voltage electrophoresis at pH 1·9 at 3 KV 100 ma for one hour. The mushroom contained thirteen free amino acids, *viz.*, serine, glutamic acid, alanine, glycine, lysine, aspartic acid, arginine, tyrosine, methionine, valine, phenylalanine, isoleucine and leucine. In addition to the above the presence of cystine and threonine was shown by faint spots. The free amino acids reported in *Pleurotus* sp. were similar except tyrosine and valine which were not detected[4].

Sensory evaluation of the recipes (*saute*) prepared from both the species showed that *P. eous* tastes softer and has stronger aroma compared to *P. flabellatus*. A freeze dried product of pink mushroom was more attractive.

The authors record their grateful thanks to Dr. D. N. Peglar, Kiew, for identification of the collected mushroom, Authors are indebted to Dr. M. V. Patwardhan and Dr. B. L. Amla, Director, Central Food Technological Research Institute, for their kind encouragement.

Fruit and Vegetable Technology Discipline,
Central Food Technological Research Institute,
Mysore 570 013, India,
May 20, 1977.

N. S. Singh.
S. Rajarathnam.

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* Personal communication.

1. Zakia Bano, and Srivastava, H. C., *Food Sci.*, 1962, 11, 363.
2. Kaul, T. N. and Janardhanan, K. K., *Indian Phytopath.*, 1970, 23, 578.
3. Rangaswamy, G., Kandaswamy, T. K. and Ramaswamy, K., *Curr. Sci.*, 1975, 44, 403.
4. Zakia Bano, Srinivasan, K. S. and Srivastava, H. C., *Appl. Microbiol.*, 1963, 11, 184.

COLURA DUM. (HEPATICAE)—A GENUS NEW TO INDIAN FLORA

Eastern Himalayas are known to harbour man interesting genera of phylogenetic significance, *viz.*, *Calobryum* Nees, *Haplomitrium* Nees, *Takak* Hattori and Inoue, etc. The present note add another rare genus *Colura* Dum. of the famil Lejeuneaceae. The occurrence of this genus i Indian Flora as *C. ornata* Goebel (*cf.* Chopra[1], Parihar[2], Kachroo[3]) is erroneus. In fac *C. ornata* is distributed in Malaya Pen., Java, Borneo, and Philippines only.

Leaves with variously shaped sacs, amphigastri doubled, are the chief distinguishing characters o the taxon. In *Colura* the leaf structure is ver complicated. The lobule is inrolled against the lobe and becomes concrescent with it, thus forming a canal leading into a sac. The sac is provided with a valvular closing apparatus, like that o *Utricularia,* allowing the water to run in, but preventing it from escaping.

Distribution

Jovet–Ast[4], in the monograph of the genu listed 51 species and remarked that the genus *Colura* may be regarded as pantropical-temperat subantarctic in distribution. Its altitudina distribution is also of interest. It ranges fro plains to height of *ca* 1,500 m. The presen report of the genus from Darjeeling Himalaya (Tiger Hill, *ca* 2573 m) now adds further to it known range of distribution.

The specimens worked out in the present stud revealed two different taxa, of which one i *C. tenuicornis* (Evans) Steph. while the other i *C. calyptrifolia* (Hook.) Dum.

Specimens examined

Tiger Hill (*ca* 2573 m) Darjeeling, Easter Himalayas, Lal and Ghosh, 205, 207; 235. 13-11-7 (in CAL), epiphyllous, on upper surface of dic leaves.

Cryptogamic Unit,
Botanical Survey of India,
Sibpur, Howrah-3, *June* 23, 1977.

Jagdish Lal.

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1. Chopra, R. S., *J. Indian bot. Soc.*, 1943, 2 237.
2. Parihar, N. S., *Univ. Allahabad Studies* (India Botany Section, 1961–62.
3. Kachroo, P., *Bull. Bot. Surv. India*, 197 12, 226.
4. Jovet–Ast, S., *Rev. Bryol. Lichenol.*; 195 22, 206; 1954, 23, 1.

6623/77. Published by S. R. S. Sastry, Manager. Current Science Association, Bangalore-560006 and Printed at. The Bangalore Press, Bangalore-560018.

THE ACTION OF ISATIN WITH REACTIVE METHYLENE GROUP: INDOLE-(3)-[2-ARYLIMINO-3-BENZYL-4-THIAZOLIDONE-(5)] INDIGOS AS FUNGICIDES

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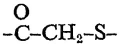
A series of Indole-(3)-[2-arylimino-3-benzyl-4-thiazolidone(5)]-indigos were synthesised by the condenation of isatin with 3-benzyl-2-arylimino-4-thiazolidones.

It has been observed that the activated methylene group of the substances having the structure  $-\overset{O}{C}-CH_2-S-$ could be readily condensed with the carbonyl group of aldehydes and ketones[1]. The methylene group[2] of the thiazolidonyl nucleus[3] has also been known to be reactive for condensation[4] with isatino carbony group. A number of derivatives of isatin- -thiosemicarbazone[5] are known to have antiviral activity. Because of the simplicity of the reaction and fungicidal activity of dyes[6] we have undertaken to prepare the Indole-(3)-[2- arylimino-3-benzyl-4-thiazolidone-(5)] indigos.

In this paper, the synthesis of these thioindigoid dyes and their elucidation of their structures have been reported. 3-Benzyl-2-arylamino-4-thiazolidones which were the starting materials were prepared by the cyclisation of N-benzyl-N′-aryl thiocarbamides and α-chloroacetic acid. The NMR, IR and analytical data are self consistent with the structures of these thiazolidones (I). These thiazolidones were condensed with equimolecular quantity of isatin in glacial acetic acid and acetic anhydride.

The assignment of the structure of dyes (II) was on the basis of the elemental analysis and spectral data. All of the compounds gave a single spot in thin layer chromatography (TLC). The IR spectrum of 3-benzyl-2-*p*-bromophenylimino-4-thiazolidone shows an absorption band for carbonyl group ($> C = O$) at 1730 cm.$^{-1}$ characteristic of lactum band whereas the IR spectrum of compound indole-(3)-[2-*p*-bromophenylimino-3-benzyl-4-thiazolidone-(5)] indigo shows,

TABLE I

Experimental data and IR peaks of Indole-(3)-[2-arylmiinno-3-benzyl-4-thiazolidone-(5)) indigos

Sl. No.	Aryl group	Molecular formula	Yield (%)	M.P. (°C)	Nitrogen (%) Found	Nitrogen (%) Calcd.	Sulphur (%) Found	Sulphur (%) Calcd.	Characteristic IR peaks (cm−1)	Rf* Values
1.	Phenyl	$C_{24}H_{17}N_3O_2S$	58	271	9·87	10·22	7·28	7·78	3185 w, 1705 s 1650 s, 1605 m	0·59
2.	*o*-Tolyl	$C_{25}H_{19}N_3O_2S$	53	282	9·53	9·88	7·15	7·53	3180 w, 1700 s. 1645 s, 1585 m	0·54
3.	*p*-Tolyl	$C_{25}H_{19}N_3O_2S$	69	288	9·62	9·88	7·41	7·53	3185 w, 1700 s 1645 s, 1590 m	0·66
4.	*o*-Chlorophenyl	$C_{24}H_{16}N_3O_2SCl$	63	296	9·19	9·42	6·94	7·18	3180 w, 1705 s. 1645 s, 1590 m	0·65
5.	*p*-Chlorophenyl	$C_{24}H_{16}N_3O_2SCl$	72	284	9·06	9·42	6·82	7·18	3185 w, 1705 s, 1650 s, 1595 m	0·66
6.	*o*-Methoxyphenyl	$C_{25}H_{19}N_3O_3S$	49	273	9·31	9·52	7·05	7·26	3190 w, 1700 s, 1665 s, 1625 m	0·62
7.	*p*-Methoxyphenyl	$C_{25}H_{19}N_3O_3S$	57	268	9·16	9·52	6·88	7·26	3180 w 1705 s, 1650 s, 1585 m	0·60
8.	*o*-Ethoxyphenyl	$C_{26}H_{21}N_3O_3S$	43	221	8·89	9·23	6·25	7·03	3180 w, 1700 s, 1650 s, 1508 m	0·59
9.	*p*-Ethoxyphenyl	$C_{26}H_{21}N_3O_3S$	67	234	9·05	9·23	6·55	7·03	3180 w, 1700 s, 1640 s, 1565 m	0·63

* R_f values were measured on developing the TLC plates (adsorbent, silica gel BDH) in benzene ether (3:1) mixture.

characteristic absorption bands at 3170 cm–1, 1720 cm–1 and 1700 cm–1 for > NH, > C = O and > C = O groups respectively. The presence of additional bands, one at 3170 cm–1 in > NH region and another at 1700 cm–1 in carbonyl region clearly indicate the attachment of the isatino carbonyl group with 5-position of the thiazolidonyl nucleus.

EXPERIMENTAL

All melting points were taken by capillary method and are uncorrected. The purity of the products was tested by thin layer chromatography (TLC). *Varian A60 D* model was used for recording of NMR spectra, *Perkin-Elmer* 257 for IR and a *Coleman Analyzer* for analyses.

3-*Benzyl*-2-*p*-*bromophenylimino*-4-*thiazolidone*—A mixture of N-benzyl-N′-*p*-bromophenyl thiocarbamide (16·05 g), monochloroacetic acid (5 g), fused sodium acetate (8 g) and absolute alcohol (50 ml) was refluxed on a water-bath for 14–16 hours. After distilling off ethanol, the substance was washed with hot water when a gummy product was obtained. It was solidified on keeping overnight in contact with cold water and recrystallised from ethanol, yield 89%, mp 103°. TLC: $R_f = 0·27$ (Benzene-Ether, 3 : 1). *Anal*. Calcd. for $C_{16}H_{13}N_2OSBr$: N, 7·75; S, 8·86. Found: N, 7·61; S, 8·78%. IR ν_{max}^{nujol} cm–1: 1730s, 1635 s, 1590 s, 1495 s. NMR ($CDCl_3$): 3·80 (2H, s) for methylene protons of the thiazolidonyl nucleus, 5·10 (2H, s) for benzyl methylene protons and 7·35 for aromatic protons (9H, *m*).

Similarly, other required 3-benzyl-2-arylimino-4-thiazolidones were prepared by using their respective thiocarbamides.

Indole-(3)-[2-*p*-*bromophenylmino*-3-*benzyl*-4-*thiaz done*-(5)] *indigo*—A mixture of 3-benzyl-2-*p*-brom phenylimino-4-thiazolidone (3·6 g), isatin (3 g), a hydrous sodium acetate (5 g), acetic acid (35 ml), an acetic anhydride (2 ml) was refluxed on an oil-ba for 3–4 hrs at 150–160° C. The reaction mixture w poured into excess of water. It was filtered, washe with water, dilute acetic acid and finally with ethan It was recrystallised from acetic acid, yield 87%, m 279°. TLC: $R_f = 0·68$ (Benzene-Ether 3 : 1). *An* Calcd. for $C_{24}H_{16}N_3O_2SBr$: N, 8·57; S, 6·53. Foun N, 8·37; S, 6·29%. IR ν_{max}^{nujol} cm–1: 3170 1720 m, 1700 s, 1640 s and 1585 s.

Likewise, other indole-(3)-[2-arylimino-3-benzyl thiazolidone-(5)] indigos were prepared. Their yiel melting points, R_f values, analytical and spectral d are recorded in Table I.

The screening programme of these compounds in progress.

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1. Hil, J. and Henze, H. R., *J. Am. Chem. S* 1924, **46**, 2806.
2. Bhargava, P. N. and Chaurasia, M. R., *J. Pha Sci.*, 1969, **58**, 896.
3. – and Kaul, C. L., *J. Indian Chem. Soc.*, 1 **49**, 32.
4. Rao, R. P., *Chem. Ber.*, 1959, **92**, 2600.
5. Bauer, D. and Dadler, P. W., *Brit. J. Pharma* 1960, **15**, 101.
6. Greenberg, B. E. and Broaney, M. L., *New Eng J. Med.*, 1933, **209**, 1153.

A NON-DESTRUCTIVE METHOD FOR THE DETERMINATION OF LANTHANUM AND COPPE USING A LOW-FLUX NEUTRON SOURCE

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ABSTRACT

The possibility of using a low-flux Am^{241}-Be neutron source for the determination of lanthanum and copper by non-destructive neutron activities analysis when present together has been investigated.

LOW flux neutron sources are being used for the Neutron Activation Analysis[1–11]. A 5-Ci Am^{241}-Be neutron source employed for the analysis of important elements in various substances was employed in this work.

Since the flux of this neturon source is small (10^4 neutrons cm^{-2} sec^{-1}), one needs large quantity of sample for irradiation in order to obtain reasonable amount of radioactivity for accurate measure Large quantity of sample causes self-shielding of trons during irradiation and self-scattering and absorption of radiation during counting. In vie this difficulty, known amounts of the element t determined are added to the sample and then irr ted. This procedure limits the volume of the sa and yet produces considerable amount of radioac in the sample. In this method, the samples are irradated for a long time (6 times the half life of the isotope formed) in order to get saturation activity which is the highest activity one can get with a given neutron source. Any further delay in the measurement is of no advantage. Hence we have employed graphical method which finds application when the gamma ray peaks corresponding to isotopes of two different elements overlap in the gamma ray spectrum. We have found that the graphical method gives better results than the ordinary comparator method[11,10].

When a sample containing both lanthanum and copper is irradiated using thermal neutrons, both lanthanum and copper get activated. The nuclear data for lanthanum and copper are given in Table I.

TABLE I

Nuclear data for lanthanum and copper

Element	Isotope	% abundance	Thermal neutron cross-section (barns)	Half life of isotope formed
Cu	Cu^{63}	69·1	4·7	12·74 h (Cu^{64})
	Cu^{65}	30·9	1·9	5·1 min (Cu^{66})
La	La^{139}	99·91	9·1	40·2 h (La^{140})

La^{140} gives four peaks of energies 0·328, 0·487, 0·815 and 1·596 MeV and Cu^{64} gives two peaks of energies 0·511 MeV and 1·34 MeV. But the 1·34 MeV peak of Cu^{64} is not clearly seen in the spectrum. If NaI (Tl) crystal is used as detector the 0·487 and 0·511 Mev peaks which are due to La^{140} and Cu^{64} respectively merge together and appear as a single peak. So determination of either lanthanum or copper in substances where they are present together becomes difficult. For such cases, we have devised a method to determine both these elements simultaneously. We have used lanthanum cuprate (La_2CuO_4) as a representative example to demonstrate the applicability of our method.

EXPERIMENTAL AND RESULTS

Two sets of standard samples are prepared in such a way that the amount of La_2CuO_4 presents in each sample is the same (0·4 gm) but vary in the amounts of Cu O (0·05–0·35 gm) in one case and La_2O_3 (0·05–0·35 gm) in the other. These two sets of samples are irradiated in the thermal neutron source in the region of constant neutron flux ($\approx 10^4$ m/cm^2 – sec.) for 10 days in order that La^{140} also attains the saturation activity. However irradiation for a period of 5 days (3 half lives) can also be employed.

After irradiation, the activities of the samples are measured by means of a 400 channel gamma-ray pulse height analyser (Frieseke and Hoepfner) using a 2″× 2″ NaI (Tl) crystal as detector. The counting time is kept at 30 min in every case.

In the case of CuO addition samples, the peak fractions of the merged peak are calculated by means of Covell's method[12,8] whereas in the case of La_2O_3 addition samples, the peak fractions of both the merged peak as well as the 1·596 MeV peak of La^{140} are calculated. The peak fraction of the 1·596 MeV peak in the case of La_2O_3 addition samples is plotted against the amount of lanthanum added to the same amount of La_2CuO_4. A straight line plot (A) is obtained. The intercept of the plot "A" corresponds to the counts due to La present in La_2CuO_4. Using this intercept value, the amount of lanthanum (y) present in the La_2CuO_4 is read from plot A.

The peak fraction of the merged peak in the case of La_2O_3 addition samples, is plotted against the amount of lanthanum added. A straight line plot (B) is obtained. The intercept corresponds to the combined activity due to the lanthanum and copper present in La_2CuO_4. From this plot B, the change in peak fraction for 1 gm. of lanthanum is calculated. Similarly, the change in peak fraction per 1 gm copper is calculated from plot C which is obtained by plotting the peak fraction of the merged peak against the amount of copper added in the case of CuO addition samples. The intercept values of plots B and C should be equal.

Now, if "a" is the change in peak fraction per 1 gm of copper and "b" is that per 1 gm of lanthanum and if "x" and "y" are the amounts of copper and lanthanum respectively present in the La_2CuO_4,

$$N = a\,x + b\,y \qquad \textbf{(1)}$$

where "N" Is the intercept value obtained either from plot B or C. "a" and "b" are calculated from plots B and C respectively and "y" has been calculated from plot A. By using equation (1), the amount of copper present in La_2CuO_4 is calculated. A representative set of results obtained is given in Table II.

TABLE II

Results obtained for the determination of lanthanum and copper in La_2CuO_4

Sl. No.	% Copper		% Lanthanum	
	Theoretical	Found	Theoretical	Found
1.	15·68	15·24	68·53	69·12
2.	,,	15·63	,,	68·13
3.	,,	16·02	,,	68·9
4.	,,	15·94	,,	69·0

1. Meinke, W. W. and Anderson, R. E., *Anal. Chem.*, 1953, **25**, 778.
2. — and —, *Ibid.*, 1954, **26**, 907.
3. Kuusi, J., *Nuclear Applications and Technology*, 1970, **8**, 465.
4. Strain, J. E. and Lyon, W. S., In *Radiochemical Methods of Analysis*, IAEA, Vienna, 1965, **1**, 263.
5. Wing, Y. and Wahlgren, M. A., *Anal. Chem.*, 1953, **25**, 778.
6. Kusaka, Y., *Bull. Chem. Soc. Japan*, 1964, 3 597.
7. Ahmad, I. and Morris, D. F. C., *Radiochem Radioanal. Letters*, 1967, **16** (6), 303.
8. Rao, V. R. S. and Sudhakar, Ch. *Curr. Sci.* 1977, **46**, 11.
9. — and —, *Radiochem. and Radioanal. Lette* 1977, **28**, 5.
10. Pavaskar, P. S., Reddy, G. R. and Shankar Das, M., *BARC, Report No.* 766, 1974.
11. —, — and —, *Indian J. of Technol.*, 1972, **10**, 80
12. Covell, D. F., *Anal. Chem.*, 1959, **31**, 1785.

ZINC(II) COMPLEXES WITH NITROGEN DONOR CHELATING LIGANDS

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ABSTRACT

Complexes of zinc (II) thiocyanate with nitrogen donor chelating ligands like orthophenanthroline, 5-nitroorthophenanthroline, 2, 9-dimethylorthophenanthroline, 2, 2′-dipyridyl, and 8-aminoquinoline have been synthesized and characterized by elemental analysis, infrared spectra and conductance measurements. Although in the synthesis of the complexes 1 : 1 metal : ligand ratio was maintained all the ligands form 1 : 2 metal complexes of the type Zn $(NCS)_2$ $(AA)_2$ except 2, 9-dimethylorthophenanthroline which forms 1 : 1 complex, Zn $(NCS)_2$ (2, 9-dimethylorthophenanthroline). The complexes are insoluble in common non-coordinating solvents and do not melt sharply. The infrared spectral data indicate the presence of bridging thiocyanate bonding. The complexes are soluble in coordinating solvent, dimethylsulfoxide and exhibit molar conductance values attributable to 1 : 1 electrolyte. The breaking of the polymeric structure occurs in dimethylsulfoxide and formation of complexes of the type [Zn (NCS) (DMSO) 2, 9-dimethylorthophenanthroline)] NCS and [Zn (NCS) (DMSO) $(AA)_2$] NCS is suggested. The reaction of zinc (II) thiocyanate with ethanolic solution of biguanide leads to the isolation of bis (biguanide) zinc(II).

INTRODUCTION

THE ligands orthophenanthroline, 5-nitroorthophenanthroline, 2,9-dimethylorthophenanthroline, 2,2′-dipyridyl and 8-aminoquinoline contain N–C–C–N chelating group in common and are known to form mono, bis and tris complexes with metal ions[1,2]. In the literature there is no record of mono complexes of these ligands with zinc(II) thiocyanate and the work described here deals with such complexes. The presence of thiocyanate groups in the complexes gives an added opportunity to find out the nature of bonding of the thiocyanate groups in the complexes. We reacted zinc(II) thiocyanate and the above heterocyclic amine ligands in 1 : 1 ratio in ethanolic medium, and, only in the case of 2,9-dimethylorthophenanthroline, we were able to prepare the desired mono complex. In all other cases (orthophenanthroline, 5-nitroorthophenanthroline, 2, 2′-dipyridyl and 8-aminoquinoline) we obtained bis complexes. A similar reaction with biguanide $[H_2N-C(=NH)-NH-C(=NH)-NH_2]$ led to the isolation of bis (biguanide) zinc(II). The complexes have been characterized by elemental analysis, conductance and infrared spectral measurements.

EXPERIMENTAL

Materials and Methods

Zinc (II) thiocyanate and 8-aminoquinoline were obtained from Pfaltz and Bauer Inc. (U.S.A.). Orthophenanthroline, 5-nitroorthophenanthroline, 2,9-dimethylorthophenanthroline and 2, 2′-dipyridyl were supplied by G. Frederick and Smith Chemical Co. (U.S.A.). Biguanide was purchased from Aldrich Chemical Co. (U.S.A.). Zinc was estimated complexometrically with EDTA after igniting the complexes to ZnO and dissolving ZnO in dilute HCl. Carbon, hydrogen and nitrogen were determined microanalytically. Conductance measurements (25° C) were done in DMSO with Radelkis type OK 102/1 conductivity bridge and a dip type cell which was calibrated with KCl solutions. Infrared spectra were recorded in KBr pellets on Perkin Elmer Model 21 and 237 infrared spectrophotometers and

TABLE I

The analytical and conductance data of zinc(II) complexes[a]

Complex	Stoichiometry	%Zn	%C	%H	%N	Λ_M ohm^{-1} cm^2 mole^{-1}
$Zn(NCS)_2$ (ophen)$_2$	$ZnC_{26}H_{16}N_6S_2$	Found: 11·8	57·3	3·3	15·8	70·0
		Reqd. : 12·01	57·67	2·96	15·53	
$Zn(NCS)_2$ (5-nitro-phen)$_2$	$ZnC_{26}H_{12}N_6O_4S_2$	Found: 10·7	51·1	2·7	17·2	58·2
		Reqd. : 10·30	49·44	2·22	17·74	
$Zn(NCS)_2$ (2, 9-dimephen)	$ZnC_{16}H_{12}N_4S_2$	Found: 16·4	49·8	3·7	14·0	50·0
		Reqd. : 16·71	49·36	3·08	14·39	
$Zn(NCS)_2$ (dipy)$_2$	$ZnC_{22}H_{16}N_6S_2$	Found: 13·2	53·9	3·8	17·4	66·5
		Reqd. : 13·18	53·55	3·24	17·04	
$Zn(NCS)_2$ (8-amq)$_2$	$ZnC_{20}H_{16}N_6S_2$	Found: 13·4	51·5	4·0	17·9	60·4
		Reqd. : 13·90	51·17	3·41	17·91	
Zn (biguanide)$_2$	$ZnC_4H_{12}N_{10}$	Found. 25·0	18·7	4·8	52·9	..
		Reqd. : 24·53	18·11	4·53	52·83	

[a] Abbreviations: ophen = orthophenanthroline; 5-nitrophen = 5-nitroorthophenanthroline; 2,9-dimephen = 2,9–dimethylorthophenanthroline, dipy = 2, 2′-dipyridyl; 8-amq = 8-aminoquinoline.

each spectrum was calibrated with a polystyrene film.

General Method of Preparation of the Complexes

Zinc(II) thiocyanate (0·003 mole) was dissolved in 15 ml 95% ethanol and was filtered. To the filtrate an ethanolic (95%) solution of the appropriate ligand (0·003 mole in 20 ml) was added. The mixture was refluxed for 30 min. The separated precipitates were filtered, washed with 95% ethanol and dried under vacuum at room temperature. Yield = 40–60%.

The analytical and conductance data of the complexes are given in Table I. The infrared spectral data of the complexes are presented in Tables II and III.

TABLE II

Infrared spectral data of zinc(II) complexes[a]

	ν (C–N) str.	ν (C–S) str.	ν N–C–S bend
Zn $(NCS)_2$ (ophen)$_2$	2060s	792 m 752 m	480 m
Zn $(NCS)_2$ (bipy)$_2$	2060 s	798 m	478 m
Zn $(NCS)_2$ (nitrophen)$_2$	2055 s	750 m	482 m
Zn $(NCS)_2$ (2, 9-dimephen)	2060 s	785 m	483 m 450 m
Zn $(NCS)_2$ (8-amq)$_2$	2078 s	785 m	465 m

[a] Abbrevations: s, strong; m, medium. All bands are in cm^{-1}.

RESULTS AND DISCUSSION

The complexes are synthesized by the reaction of zinc(II) thiocyanate and bidentate ligands in ethanol. The analytical data indicate that 1 : 1 complex is formed in case of 2, 9-dimethylorthophenanthroline. In the case of the other heterocyclic amines, *viz.*, orthophenanthroline, 5-nitroorthophenanthroline, 2, 2′-dipyridyl and 8-aminoquinoline, 1 : 2 complexes of the type $Zn(NCS)_2(AA)_2$ (where AA = heterocyclic amine) are formed. The complexes are insoluble in common non-coordinating organic solvents and conductance measurements in non-coordinating solvents were not possible. However, the complexes are soluble in coordinating solvents like dimethylsulfoxide (DMSO) and we recorded the conductance of the complexes in 0·001 M solution in DMSO. The complexes are not soluble beyond the concentration 0·001 M in DMSO at 25° C. The molar conductance values of the complexes are in the range 50–70 ohm^{-1} cm^2 mole^{-1} in DMSO. The values are indicative of 1 : 1 electrolyte in this solvent[8]. The insolubility of the complexes in common solvents and failure to melt sharply give an indication of polymeric nature of the complexes,

TABLE III

Infrared spectral data of biguanide and Zn (biguanide)$_2$

Biguanide	Zn (biguanide)$_2$	Possible assignments
3300 s	3150 s	ν (NH$_2$)
1625 s	1644 s	NH def.
1540 s 1420 s	1512 s 1368 s	N–C–N str.
	1252 s	ring vibration
1200 s	1108 w	δ (NH) and ν (CN)
1100 s		δ (NH) and ν (CN)
1030 m	1062 m	ν (CN)
915 w	945 s	ν (CN)
	895 s	
	765 w	
	738 w	π (NH)
655 w	695 w	π (NH) and δ(N–C–N)

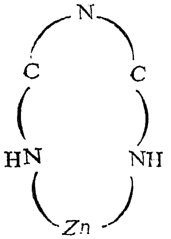
Infrared spectral data of the complexes (discussed later) indicate the presence of bridging thiocyanate groups. In coordinating solvent DMSO, the polymeric nature of the complex is broken and presumably the complexes of the type [Zn(NCS) (DMSO) (2,9-dimethylorthophenanthroline)] NCS and [Zn(NCS) (DMSO) (AA)$_2$] NCS (where AA = orthophenanthroline, 5-nitroorthophenanthroline, 2,2′-dipyridyl and 8-aminoquinoline are formed.

The ambient nature of thiocyanate ion is well established[4,5]. Zinc(II) ion being intermediate in class *a*-class *b* behaviour, is able to bind to either S or N atoms, depending upon the other ligands present in the molecule. The thiocyanate ion has three fundamental infrared absorption frequencies : the strong band due to the C–N stretch, the weak C–S stretch and the weak N–C–S bend. Infrared spectra have been claimed to indicate the linkage of the thiocyanate groups with the metal ions and a correlation of the thiocyanate bonding type with ranges of the thiocyanate fundamental frtquencies is summarized below[6,7]. The infrared data of the complexes of zinc(II) thiocyanate with heterocyclic amines indicate that the thiocyanates are bridged in a polymeric structure[6,7]. The physical properties, such as insolubility in common non-coordinating solvents and failure to melt sharply also indicate polymeric nature of the complexes. Our complexes do not have C–S stretching frequency below 750 cm^{-1} and this is indicative of absence of a thiocyanate S-bonded structure. We believe our complexes are thiocyanate bridged polymeric chains with stronger N– than S–bonding[7].

	ν (C–N) cm^{-1}	ν (C–S) cm^{-1}	ν (N–C–S) cm^{-1}
NCS	2053	746	486, 471
M–NCS	2050–2100	820–870	475–485
M–SCN	2085–2130	700–760	430–470
M–NCS–M	2045–2165	750–800	440–480

The intent of this research was to prepare 1 : 1 complexes of zinc(II) thiocyanate and bidentate NN donor ligands. Only in one case, *viz.*, Zn(NCS)$_2$ (2, 9-dimethylorthophenanthroline) 1 : 1 complex was obtained and with all other ligands 1 : 2 complex, Zn(NCS)$_2$ (AA)$_2$ was isolated. The unusual behaviour of 2,9-dimethylorthopheannthroline, can be explained if one considers the steric hindrance of methyl groups in 2, 9-dimethylorthophenanthrolines[8].

The treatment of alcoholic solution of biguanide with alcoholic solution of zinc(II) thiocyanate led to the synthesis of Zn(biguanide)$_2$. The infrared spectral data of biguanide and Zn(biguanide)$_2$ are given in Table III along with the possible band assignments. The N–C–N stretch of biguanide at 1540, 1420 cm^{-1} is markedly lowered in the complex. The NH def. vibration of biguanide is higher in Zn(biguanide)$_2$. This shift of the infrared bands is consistent with the nitrogen coordination of the ligand biguanide. A strong band at 1252 cm^{-1} in Zn(biguanide)$_2$ is assigned to chelate ring vibration of the following fragment of the molecular structure[9]. This band is absent in the infrared spectrum of biguanide.



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1. Brandt, W. W., Dwyer, F. P. and Gyarfas, E. C., *Chem. Rev.*, 1954, **54**, 960.
2. Sutton, G. J., *Aust. J. Chem.*, 1961, **14**, 37; 550; 1962, **15**, 232, 563; 1963, **16**, 1134.
3. Geary, W. J., *Coord, Chem. Rev.*, 1971, **7**, 81.
4. Ahrland, S., Chatt, J. and Davis, N. R., *Quart. Revs.*, 1958, **12**, 265.
5. Burmeister, J. L., *Coord. Chem. Rev.*, 1966, **1**, 205; Norbury, A. H. and Sinha, A.I.P., *Quart. Revs.*, 1970, **24**, 69.
6. Norbury, A. H., *Advances in Inorganic Chemistry and Radio-Chemistry*, 1975, **17**, 231.
7. Baranyi, A. D., Makhija, R. and Onyszchuk, M., *Can. J. Chem.*, 1976, **54**, 1189.
8. Cotton, F. A. and Wilinson, G., *Advanced Inorganic Chemistry*, 2nd Edition, Wiley Eastern, 1972, p. 156.
9. Kedzia, B. B., Armendarez, P. X. and Nakamote, K., *J. Inorg. Nucl. Chem.*, 1968, **30**, 849.

SYNTHESIS AND STRUCTURAL STUDIES OF SOME FIRST ROW TRANSITION METAL COMPLEXES OF ISONICOTINIC ACID HYDRAZIDE

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ABSTRACT

Isonicotinic acid hydrazide (INH) complexes of the types M (INH) $Cl_2 \cdot n$EtOH]where M= Mn(II), Fe(II), Co(II), Ni(II) and Cu(II) and $n = 0$ or 1] M $(INH)_2$ $(NO_3)_2$ [where M= Co(II) and Ni(II)] and mixed metal complexes like $HgCo_2$ $(INH)_2Cl_6$ and $Ni(INH)_2$ HgI_4 have been prepared and their nature and structure studied by molar conductance, magnetic susceptibility, electronic, E.S.R. and i.r. spectral measurements. Spin-free octahedral geometry has been proposed for all the complexes except Mn(INH) Cl_2, Co(INH) Cl_2 and $HgCo_2$ $(INH)_2$ Cl_6 for which tetrahedral geometry has been suggested around Mn(II) and Co(II) metal ions.

INTRODUCTION

IN continuation of our earlier work[1] on the complexes of titanium and zirconium tetrahalides with isonicotinic acid hydrazide (INH), we report here the results of our studies on Mn(II), Fe(II), Co(II), Ni(II) and Cu(II) complexes of INH.

EXPERIMENTAL SECTION

Starting Materials

All the chemicals used in this study were of BDH or equivalent quality. Ferrous chloride was freshly prepared as described by Mellor[2]. INH, supplied by Schuchardt Chemical Company, West Germany, was used as such.

Synthesis and Analysis of the Complexes

$Fe(INH)Cl_2$.EtOH was prepared under hydrogen atmosphere by mixing together cold ethanolic solutions of the metal chloride and INH in *ca* 1 : 2 molar ratio. The precipitated complex was filtered in a specially designed vacuum filtering unit, washed with ethanol and dried *in vacuo*. Mn(II), Co(II), Ni(II) and Cu(II) complexes were prepared by mixing the hot ethanolic solutions of the metal chloride/nitrate and INH in *ca* 1 : 2 molar ratio. $HgCo_2(INH)_2Cl_6$ was prepared by adding *ca* 0·02 mole of cobalt chloride to a mixture of 0·01 mole of mercuric chloride and 0·02 mole of INH in ethanol. $[Ni(INH)_2]HgI_4$ was prepared by adding successively *ca* 0·02 mole of INH and 0·01 mole of nickel nitrate to *ca* 0·01 mole of K_2HgI_4 in aqueous medium. The complexes, thus obtained, were filtered, washed with ethanol/water and dried at room temperature.

All the complexes were analysed for metal using standard procedures[3] while nitrogen, hydrazine, chloride and ethanol contents were estimated as described earlier[4]. Nitrate was determined gravimetrically using nitron reagent. The experimental details pertaining to the molar conductance, magnetic susceptibility, i.r., electronic and e.s.r. spectral measurements were the same as described in our earlier paper[4]. The analytical and electronic spectral data are given in Tables I and II respectively.

RESULTS AND DISCUSSION

The analytical data (Table I) show that INH forms two types of complexes with metal chlorides and nitrates having 1 : 1 and 1 : 2 metal to ligand ratios respectively. All the complexes do not melt upto 250° C. They are insoluble in common organic solvents but with the exception of $Ni(INH)Cl_2$.EtOH, all of them are fairly soluble in DMSO. The non-melting nature and their

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TABLE I

Analytical data and general behaviour of INH complexes

Complex	Colour	Metal %	Chloride/ nitrate %	Hydrazine %	Loss of wt. %	μ_{eff} (B.M.)	Molar conductance (mhos cm²/mole)
$Cu(INH)Cl_2 . EtOH$	Light green	19·8 (20·0)	22·05 (22·3)	13·1* (13·2)	13·9 (14·5)	1·98	3·4
$Ni(INH)Cl_2 . EtOH$	Light green	18·65 (18·8)	22·5 (22·7)	10·0 (10·2)	14·2 (14·7)	3·15	..
$[Ni(INH)]_2 (NO_3)_2$	Light blue	12·8 (12·9)	26·85 (27·1)	24·3* (24·5)	..	3·09	76·7
$[Ni(INH))_2] HgI_4$	Light green	19·15 Hg (19·3), 5·3 Ni (5·6)	..	8·15 (8·1)	..	2·97	47·8
$Co(INH) Cl_2$	Blue	21·8 (22·1)	26·5 (26·6)	11·85 (12·0)	..	4·48	5·3
$[Co(INH)_2] (NO_3)_2$	Pink	12·7 (12·9)	26·8 (27·1)	24·4* (24·5)	..	5·08	85·6
$HgCo_2 (INH)_2Cl_6$[a]	Blue	24·7 Hg (24·95)	26·25 (26·4)	7·85 (7·9)	..	4·62	5·1
$Fe(INH) Cl_2 . EtOH$	Orange red	18·2 (18·0)	22·7 (22·9)	10·2 (10·3)	14·3 (14·9)	5·46	4·0
$Mn(INH) Cl_2$	White	20·6 (20·9)	26·8 (27·0)	12·05 (12·2)	..	5·92	3·8

Calculated values are given in parentheses.
* Values indicate nitrogen analyses

[a] Value of cobalt analysis: 14·25 (14·6)

insolubility in common organic solvents indicate the polymeric nature of the complexes. The loss of ethanol molecules in Fe(II), Ni(II) and Cu(II) complexes at relatively high temperatures (130–160° C) indicates that the solvent molecules are coordinated to metal ions in these complexes. All the chloride complexes are non-ionic and the nitrate complexes are 1 : 2 electrolytes as shown by their molar conductance values (lying in the ranges 3·4–5·1 for the former type and 76·7–85·6 mhos cm²/mole for the latter) in M/1000 DMSO solution; $[Ni(INH)_2]HgI_4$ has been found to behave as 1 : 1 electrolyte (47·8 mhos cm²/mole)[5].

Magnetic Measurements

The magnetic moments (Table I) are all normal and indicate that the complexes of Fe(II), Ni(II) and the complex $Co(INH)_2 (NO_3)_2$ are all spin-free octahedral[6]. The μ_{eff} values observed for $Co(INH)Cl_2$ and $HgCo_2(INH)_2Cl_6$ suggest tetrahedral geometry[6] around Co(II) atom. A spin-free tetrahedral or octahedral geometry is suggested for the Mn(II) complex from its magnetic moment. However, from the foregoing discussion of its i.r. spectrum, spin-free tetrahedral geometry may be proposed for the complex. The μ_{eff} value corresponding to one unpaired electron in the case of Cu(II) complex does not give any information about its stereochemistry.

Electronic and e.s.r. Spectra

The positions and assignments of the bands given in Table II indicate tetrahedral geometry[7] for $Co(INH)Cl_2$ and $HgCo_2(INH)_2Cl_6$ and octahedral geometry[8] for all the complexes of Fe(II), Co(II), Ni(II) and Cu(II). These results are in accord with the magnetic moments discussed earlier. The band observed at 13330 cm⁻¹ in the spectrum of Cu(II) complex is considerably blue-shifted when compared with the first band maximum of the corresponding Ni(II) complex indicating Jahn-Teller distortion of the ^2Eg state in the Cu(II) complex. The ratio ν_{Cu}/ν_{Ni} comes out to be 1·36 indicating an appreciable distortion from octahedral geometry[9]. Wherever possible, ligand-field para-

TABLE II

Electronic spectral data and variou.. ligand-field parameters of INH complexes

Complex	Band maxima (cm^{-1})	Assignments	10 Dq (cm)$^{-1}$	B′ (cm^{-1})	β	β° %	LFSE (kJ/mole)
$Cu(INH)Cl_2 \cdot EtOH$	$\nu_1 = 13330$	$^2E_g \rightarrow {}^2T_{1g}$	13330	..	..	..	95·23
$Ni(INH)Cl_2 \cdot EtOH$	$\nu_1 = 9804$; $\nu_2 = 16130$	$^3A_{2g} \rightarrow {}^3T_{2g}$, $^3T_{1g}$ (F)	9804	966·5	0·91	8·47	140·20
$[Ni(INH)_2](NO_3)_2$	$\nu_1 = 10810$; $\nu_2 = 17540$	do.	10810	954·1	0·90	9·64	154·50
$[Ni(INH)_2]HgI_4$	$\nu_1 = 11111$; $\nu_2 = 17700$	do.	11111	863·4	0·82	18·24	158·80
$Co(INH)Cl_2$	$\nu_2 = 2025$; $\nu_3 = 15380$	$^4A_2 \rightarrow {}^4T_1$ (F), $^4T_1$ (P)	2811	792·4	0·82	18·05	40·17
$HgCo_2(INH)_2Cl_6$	$\nu_2 = 2040$; $\nu_3 = 15150$	do.	2790	778·8	0·80	19·46	39·87
$[Co(INH)_2](NO_3)_2$	$\nu_1 = 9434$; $\nu_2 = 19050$; $\nu_3 = 20410$	$^4T_{1g}$ (F) $\rightarrow$ $^4T_{2g}$ (F), $^4A_{2g}$ (P), $^4T_{1g}$ (P)	9616	743·9	0·77	23·08	91·62
$Fe(INH)Cl_2 \cdot EtOH$	$\nu_1 = 11760$	$^5T_{1g} \rightarrow {}^5E_g$ (D)	11760	..	..	..	56·03

meters such as 10Dq, B′, β, β° and LFSE have been calculated and listed in Table II. The 10Dq values of the complexes indicate a position between water and ammonia for the ligand under discussion in the spectrochemical series.

In order to obtain additional evidence for the proposed structure of the copper complex, its paramagnetic resonance spectrum has been recorded. The spectral features are of axial symmetry and the data, $g_{\parallel}$ (2·2662) > $g_{\perp}$ (2·0467) > free-spin (2·0023), indicate that the unpaired electron is in the $d_{x^2-y^2}$ orbital of Cu(II) atom. The values $g_{\parallel}$ and $g_{\perp}$ are close to those reported for distorted octahedral structure of Cu(II) complexes[10], supporting the conclusion drawn earlier from the electronic spectrum of the complex.

Infrared Spectra

The bonding sites of INH involved in its complexes have been determined by a careful comparison of the i.r. spectra of the complexes with the acetonitrile solution spectrum of the ligand.

The amide I, II, III and ν(N–N) bands are observed at 1670, 1560, 1328 and 982 cm^{-1} in the solution spectrum of the ligand and in 1650–1620, 1548–1540, 1340–1335 and 1020–1010 cm^{-1} regions respectively in the nujol spectra of all the complexes. A negative shift in amide I and II bands and a positive shift in amide III and ν(N–N) modes in the spectra of all the complexes compared with the spectrum of the ligand are indicative of coordination through C=O and terminal NH_2 groups of the ligand[11,12]. The in-plane and out-of-plane ring deformation modes occurring at 658 and 400 cm^{-1} in the nujol spectrum of the ligand are shifted to 700–690 and 470–460 cm^{-1} regions respectively in the spectra of all the complexes except $Mn(INH)Cl_2$ due to bonding through ring nitrogen[13]. The non-splitting of the ν_3 band of nitrate ion appearing at 1380 cm^{-1} in the spectra of the nitrate complexes indicate that the nitrate groups are ionically bonded[14]. The non-ligand bands occurring in 425–410, and 358–300 cm^{-1} regions in the spectra of all the complexes may be tentatively assigned to ν(M–O) and ν(M–N) modes[13] respectively whereas those appearing in 300–280 cm^{-1} region in the spectra of all the chloride complexes may be assigned to ν(M–Cl) modes[13].

Based on chemical composition, general behaviour and physico-chemical studies, tetrahedral geometry is proposed for $Co(INH)Cl_2$, $HgCo_2(INH)_2Cl_6$ and $Mn(INH)Cl_2$ around Co(II) and Mn(II) metal ions and polymetallic structures with octahedral environment around the metal ion are suggested for the other complexes.

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1. Aggarwal, R. C., Prasad, T. and Yadav, B. N., *J. Inorg. Nucl. Chem.*, 1975, **37**, 899.
2. Mellor, J. W., *Inorganic Synthesis*, Ed. by Therold Moeller, (McGraw-Hill), 1957. **5**, 179.
3. Vogel, A. I., *A Text-book of Quantitative Inorganic Analysis*, 3rd Ed. (Longmans, Green, London), 1961.
4. Aggarwal, R. C and Rao, T. R., *Trans. Met. Chem.*, 1977, **2**, 21.
5. Geary, W. J. *Coord. Chem. Rev.*, 1971, **7**, 81.
6. Figgis, B. N. and Lewis, J., *Progress in Inorg. Chem.*, 1964, **6**, 197.
7. Cotton, F. A., Goodgame, D. M. L. and Goodgame, M., *J. Am. Chem. Soc.*, 1961, **83**, 4690.
8. Lever, A. B. P., *Inorganic Electronic Spectroscopy* (Elsevier, Amsterdam), 1968.
9. Jorgensen, C. K., *Acta Chem. Scandinavica*, 1955, **9**, 1362.
10. Landschoot, R. C. V., Hest, J. A. M. V. and Reedijk, J., *J. Inorg. Nucl. Chem.*, 1976, **38**, 185.
11. Nagano, K., Kinoshita, H. and Hirakawa, A., *Chem. Pharm. Bull.*, 1964, **12**, 1198.
12. Braibanti, A., Dallavalle, F., Pellinghelli, M. A. and Leporati, E., *Inorg. Chem.* 1968, **7**, 1430.
13. Nakamoto, K., *Infrared Spectra of Inorganic and Coordination Compounds*, 2nd Ed. (Wiley-Interscience), 1970, pp. 152, 167, 212.
14. — and McCarthy, S. J., *Spectroscopy and Structure of Metal Chelate Compounds*, John-Wiley and Sons, N.Y., 1968, p. 265.

ANALYTICAL APPLICATIONS OF HYDROXYPYRIDINES

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ABSTRACT

Analytical applications of hydroxypyridines have been reviewed.

The chemistry of the pyridines has been extensively reviewed[1–7]. The compounds have also been used since long for analytical purpose and the literature is replete with the analytical work[8–12] on pyridine azo-dyes, pyridine carboxylic acids, bipyridyls, pyridine oximes and pyridine itself. However, simple hydroxypyridines (pyridinols) have only been recently introduced as analytical reagents. Their analytical applications are reviewed.

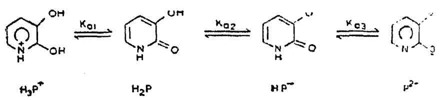
ANALYTICAL APPLICATIONS

2,3-*Dihydroxypyridine* (*DHP*)

ONE of the earliest references to DHP was to its preparation by alkali fusion of 3-hydroxypyridine[13]. Since then most of the literature on this compound has dealt only with alternative methods of synthesis[14–20] or made mention of it as a by-product of other synthetic work[21–26]. The product is now commercially available and quite inexpensive. It melts at 248° C. It has been described as being a useful intermediate in many organic syntheses[23,26], its use in the conversion of tetrahydrofurans into quaternary ammonium compounds having antibacterial action[37]. There are other reports of the biological importance of DHP. In 1956 it was mentioned with other pyridinediols in a paper on compounds active against salmonelladysentery group bacilli[28]. It has also been described as an important intermediate in the bacterial metabolism of the pyridine ring[29].

Spinner and White studied the spectra and ionization constants of substituted 2-hydroxypyridines[30] including DHP and those of the cations and anions of the compounds[31]. The acid dissociation constants of the cationic and neutral species of DHP at 25° C have been determined more precisely and in concentration terms[32,33].

The stepwise deprotonation of DHP takes place as shown below:



The overall stability constant of the complex with iron(III) in 1·0 M HCl has been evaluated. The composition of the complex appears to be FeClHP and the value of the conditional stability constant comes out to be 12·13 with a standard deviation of 0·06.

Stability constants of 1 : 1 bivalent metal complexes with DHP were determined[34,35] in aqueous and 50% dioxan media using the potentiometric method. They follow the order :

$UO_2 > Cu > Pb > Zn > Ni > Co > Cd > Mn$

in agreement with the general Irving-Williams order of stability of metal complexes[36]. A similar order in which Zn > Ni was observed for catechol and *o*-diehydroxy ligands.[37–40]. The stability constants were also determined[34,35] for rare earth complexes. The order is

$Yb > Er > Gd > Sm > Nd > Pr > Co > La.$

The stability of metal complexes increases with increasing atomic number and decreasing ionic radii of the rare earth metals. A similar order of stability of rare earth complexes was observed with iron[41]. Many ligands other than *o*-dihydroxy compounds also follow the same order[42].

DHP and other ortho and para-dihydric phenols have been colorimetrically determined in presence of monohydric phenols[43]. Reio reported chromatographic data in six solvent systems and colour reactions with several reagents including DHP[44]. The colour reactions of dihydroxypyridines with iron(III) chloride have been known for many years. In 1928 when deep blue colours were noted to result from the reactions in faintly acid solutions, they appear as already standard qualitative tests. Later workers have used the reactions for the same purpose[45,46], the latter reference reporting the colour reactions of all six isomers of dihydroxypyridine with ferric chloride as shown below:

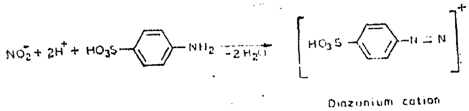
Isomer :	2,3,	2,4	2,5	2,6
Colour :	Blue	Brown-red	Pink-red	Sepia ppt
Isomer :	3,4	3,5		
Colour :	Violet	Brown-red		

The reagent was first applied to spectrophotometric determination[47] of iron (III) in 1968. Its methanolic solution absorbs maximum at 240 nm (ϵ 4·4 × 10^3) and 298 nm (ϵ 7·5 × 10^3). With iron(III), it forms two complexes depending on the acidity of the solution. The blue complex (Fe-DHP ratio 1 : 1) absorbs maximum at 550 nm. In 0·6 N $HClO_4$ medium, it obeys Beer's law from 0·5–12·0 ppm of iron. The red complex (λ_{max} 500 nm) formed at higher pH, contains metal and the ligand in 1 : 2 ratio and obeys Beer's law up to 5·0 ppm of iron.

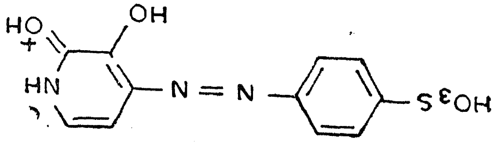
Iron(III) complexation with DHP was also investigated[48–50] in chloride, perchlorate, sulphate and sulphate-perchlorate media with a view to determine the metal more selectively. From the application of Varga and Veatch procedure[51] to estimate the number of absorbing species present in different media, it was concluded that the species contributing to absorbance contained metal and the ligand in 1 : 1 ratio. The perchlorate medium (1 M) gives the greatest molar absorptivity, 1·543 × 10^3, corresponding to 0·036 μg of iron(III) per ml for an absorbance of 0·001 in a 1 cm path.

The reagent was also applied in spot test detection of iron on filter paper[52,53] and in conjunction with ring-oven colorimetry[54]. Large number of cations and anions do not interfere. The method finds application in detection of the metal in alloys and pharmaceutical preparations. The colour of iron(III) and vanadium(IV) chelates of DHP are discharged by EDTA; the two metals can be determined chelatometrically[55,56]. The iron(III)-complexation being pH dependent, it finds use in acid-base titrations as well[56]. The end-point is sharp and reproducible within ± 0·2%. The apparent indicator constant, pK_i, is 2·3, which limits use of the indicator (iron-complex with DHP) to titrations of acids (or bases) that are $\geqslant$ 0·1 N. The indicator solution is stable for several weeks. The formation of an equimolar Os(VIII)-DHP complex is used in micro-detection[51] and spectrophotometric determination[58,59] of the metal at pH 8·5–11·0.

When NO_2^- ion interacts with sulphanilic acid, the diazenium cation results. The cation when coupled with DHP results into the formation of a red dye (I). This forms the basis of detection and determination of nitrite[60]. The red azo-dye has been further used in chelatometric determination of Zn(II), Cd(II) and Hg(II)[61].



(I)



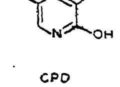
In a more recent work the complexation of Cd(II) and Pb(II) with DHP has been investigated polarographically[62]. Both form 1 : 1 and 1 : 2 (metal : ligand) complexes. In polarographic reduction of Mo(VI) with DHP, the diffusion controlled irreversible cathode waves are obtained[63].

5-Chloro-2,3-Pyridinediol (CPD)

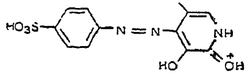
Analytical potentialities of related compound, *viz.*, 5-chloro-2, 3-pyridinediol (CPD) have also been explored[56,58–61,64]. The reagent absorbing maximum at 245 and 305 nm with corresponding molar absorptivities 4·4 × 10^3 and 6·1 × 10^3, behaves more or less similar to its precursor DHP. Addition of a few drops of an ethanolic solution of CPD to an acidic ferric solution results in a blue complex which changes to red at higher pH (boric acid-sodium hydroxide buffer). The amounts of foreign ions (μg/ml) tolerated in estimation of 2·5 μg of iron per ml in 0·1 M perchloric acid medium are: 500 of CH_3COO^-, NO_2^-, NO^{3-}, Cl^-, Br^-, SO_4^{2-}, citrate, tartrate or BO_3^{3-}; 250 of Ag^+, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+}, Hg^{2+}, Al^{3+}, Pb^{2+}, Bi^{3+} or Mn^{2+}; 150 of Cu^{2+}, UO_2^{2+}, Co^{2+} or Ni^{2+}; 50 of Th^{4+}, As^{3+}, Sb^{3+} or Sn^{4+}; 20 of PO_4^{3-} or Pd^{2+}; 10 of F^-, $C_2O_4^{2-}$ or Zr^{4+}. I^-, IO_3^-, $S_2O_3^{2-}$, EDTA, Cr^{3+} and Mo^{6+} interfere. The red complex gives a rather more selective and sensitive determination of iron. The amounts of foreign ions (μg/ml) tolerated in estimation of 2·5 μg of iron per ml at pH 9 are: 500 of CH_3COO^-, NO_2^-, NO_3^-, Cl^-, Br^-, I^-, IO_3^-, SO_4^{2-}, tartrate, Sr^{2+} or Ba^{2+}; 250 of $S_2O_3^{2-}$, PO_4^{3-}, Ag^+ or Ca^{2+}; 200 of Al^{3+}, As^{3+}, Sb^{3+}, or Sn^{4+}; 100 of citrate, Pb^{2+} or Mn^{2+}; 50 of F^-, Zn^{2+}, Hg^{2+}, Th^{4+}, Bi^{3+}, Mo^{6+}, Co^{2+}, Ni^{2+} or Pd^{2+}; 25 of $C_2O_4^{2-}$, Cu^{2+} or Zr^{4+}. The presence of EDTA, Cr^{3+} or UO_2^{2+} interferes. Both the complexes (blue and red) are easily decomposed by EDTA; this fact is utilised in chelatometric determination of iron at pH 1·4–3·2.

The iron(III)-CPD system, used as an acid-base indicator,[56] has a $pK_i = 2·8$, and is less stable than the DHP indicator.

The formation of an equimolar Os(VIII)-CPD complex has been used[58,59] in determination of the metal at pH 9·0–11·0 using the wavelength 500 nm. The red azo-dye (II) produced on interaction of NO_2^- with sulphanilic acid and CPD is used in spot test detection of the anion and its determination in water samples[60]. The dye acts as an indicator in EDTA titrations of bivalent zinc, cadmium and mercury.



CPD



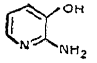
(II)

The stability constants of complexes formed by CPD with bivalent metals were determined[64] in 50% dioxan medium, the method of Bjerrum and Calvin as modified by Irving and Rossotti[65] being used to obtain values of *n* and pL which were plotted to obtain the formation curves. K_1 and K_2 were calculated by the correction-term method of Irving and Rossotti[66]. From the log K_1 values of the complexes formed by metal ions having $3d^5$–$3d^9$ configurations, the δH values were calculated according to the method described by George and McClure[67].

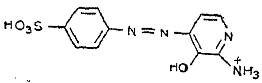
2-*Amino-3-Hydroxypyridine* (*AHP*)

AHP characterized by its λ_{max} at 235 nm (ε 5·8 × 10^3) and 300 nm (ε 6·6 × 10^3) undergoes complexation with iron(III) but the complexes are not very stable and the absorbance varies with time[56]. It, however, reacts with ruthenium (III),[68] rhodium (III)[58], osmium(VIII)[69] and iridium(IV)[58] under suitable conditions and the metals are determined spectrophotometrically.

The diazonium cation resulting on interaction of NO_2^- ion with sulphanilic acid further reacts with AHP giving the red azo-dye(III). The method is applied



AHP



(III)

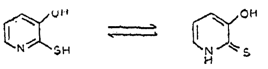
to detect and determine nitrite[60] in water samples from wells and pumps. The dye absorbs maximum at 470–485 nm (ε 2·8 × 10^4) at which wavelength the anion is determined. It is further used[61] in chelatometric titrations of Zn(II), Cd(II) and Hg(II).

Stability constants of bivalent metals with AHP, in aqueous and in 50% dioxan medium, have been reported[34]. The stability follows the order:

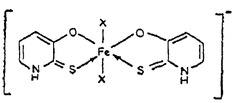
$$UO_2 > Cu > Be > Pb > Zn > Ni > Mn.$$

3-*Hydroxypyridine-2-thiol* (*HPT*)

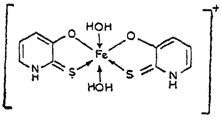
HPT absorbs maximum at 275 nm (ε 9·1 × 10^3, and 360 nm (ε 1·1 × 10^3). It reacts with iron(III), forming three complexes—green (up to pH 3·0). blue (pH 3·0–11·0) and red (pH 11·0). The green complex contains the reactants in 1 : 1 molar ratio up to pH 1·0 and 1 : 2 (iron : HPT) at higher pH. The characteristics of the complexes and their use in spectrophotometric and chelatometric determination of iron(III) have been described[64,70]. HPT also forms complexes with Pd(II)[56] and Os(VIII)[71] under suitable conditions.



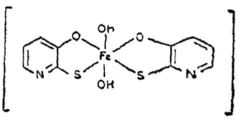
HPT



GREEN COMPLEX



BLUE COMPLEX



RED COMPLEX

The two metals are determined spectrophotometrically.

Stability constants and δH values for some bivalent metal complexes of HPT were determined potentiometrically[64] in 50% aqueous dioxan. The values of stability constant follow the general Irving William order.

3-*Nitroso*-2,6-*pyridinediol* (*NPD*)

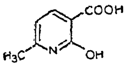
NDP was first synthesized[72] in 1916 and its reactions with NH_4^+, K^+, Rb^+ and Ba^{2+} were reported by Titov[73]. McDonald *et al.*[74–76] studied its coloured complexes with Au^{3+} (blue), Ru^{3+} (purple), Rh^{3+} (yellow), Pd^{2+} (yellow) and Os^{8+} (purple). Ru is distilled as tetraoxide prior to its determination to avoid interference from transition and other platinum group metals.

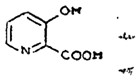
Ethyl-2,4-*dihydroxy*-3-*nitrosonicotinate* (*EDHNN*)

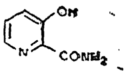
EDHNN was prepared[77] by introsating ethyl-2,4-dihydroxynicotinate and used[77] for the colorimetric determination of iron(II) at pH 2·2–4·1. The iron complex contains metal and the ligand in 1 : 1 ratio and absorbs maximum at 653 nm (ε 1·3 × 10⁴). The reagent is poor in selectivity; the transition metals interfere seriously with the estimation.

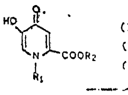
Hydroxypyridine acids and amide

The characteristics of iron(III) complexes with 6-methyl-2-hydroxypyridine-3-carboxylic acid (MPC)[78] and 3-hydroxypyridine-2-carboxylic acid (HPC) and also its amide (HPA)[56] have been studied spectrophotometrically. All the three compounds find use in micro determination of iron. The tolerance limits of diverse ons have been reported.


MPC


HPC


HPA


(IV): $R_1 = C_6H_5$, $R_2 = CH_3$; (V): $R_1 = C_6H_5$, $R_2 = C_2R_5$
(VI): $R_1 = 4-CH_3\ C_6H_4$, $R_2 = C_2H_5$
(VII): $R_1 = 4-Br\ C_6H_4$, $R_2 = C_2H_5$

5-*Hydroxy*-1-*aryl*-4-*pyridone*-2-*carboxylic acid esters*

5-Hydroxy-1-aryl-4-pyridone-2-carboxylic acid esters (IV–VII) have been prepared, characterised[79–81] and their analytical potentialities explored. The compound VI, *viz.*, 2-carbethoxy-5-hydroxy-1-(4-tolyl)-4-pyridone, abbreviated as HA, has been investigated more extensively for extraction, separation and determination of several metals[82–88]. Its pK value has been found to be 7·46 ± 0·03 (spectrophotometrically) and 8·53 ± 0·06 (potentiometrically). The difference of about 1 pK unit is attributed to the presence of 50% ethanol in potentiometric titration. The partition K_d of the reagent between chloroform and 1 M perchlorate solution was determined at constant ionic strength (0·1 M $HClO_4$ + 0·9 M $NaClO_4$) and the value of log K_d found to be 2·67 ± 0·05.

The extraction studies of niobium(V) and tantalum(V) from oxalate solution show that they can be separated from each other using HA in chloroform[83,84]. At low oxalic acid concentrations 93% of Nb present was extracted. With increasing concentration of the acid, the extraction decreased and was negligible at 5 × 10⁻¹ M oxalic acid. The behaviour of Ta differed from that of Nb. It has been possible to achieve good separation (separation factor ∼4) of Ta from Nb keeping oxalic acid concentration > 3 × 10⁻¹ M. In another study[85] the extraction and separation of Zr, Nb and Ta from oxalic and hydrofluoric acid solutions have been investigated. The following mechanism is suggested for the extraction of Zr from oxalate solution and of Nb from fluoride solution:

$$[Zr\,(C_2O_4)_4]^{4-} + 3HA \rightleftharpoons Zr\,(C_2O_4)\,A_2.HA + 3C_2O_4^{2-} + 2H^+$$

$$[NbOF_4]^- + 2HA = NbOFA_2 + 3F^- + 2H^+$$

Under appropriate conditions, Zr and Nb (in oxalate or fluoride solution) and also Nb and Ta (in fluoride solution) can be separated. The metals can be back-extracted from the organic phase with oxalic or hydrofluoric acid. The efficiency of extraction by HA decreases in the sequence Ta > Nb > Zr.

Vanadium(V) in aqueous HCl, HNO_3 or H_2SO_4 solution is extracted with HA (in $CHCl_3$) and the metal determined spectrophotometrically[86]. Depending on the composition of the aqueous phase, two complexes are formed. At pH 1 (HCl, HNO_3 or H_2SO_4), $VO_2A\,(HA)_2$ is formed with maximal absorption at 478 nm. From 4–5 N HCl, $VO_2Cl\,(HA)_3$ is extracted (λ_{max} 615 nm). The same complex is extracted from 2–3 N HNO_3 or 6–8 N H_2SO_4 in presence of enough chloride. The extraction takes place through following mechanism:

$$VO^{2+}{}_{2(aq)} + 3HA_{(o)} \rightleftharpoons VO_2A\,(HA)_{2(o)} + H^+{}_{(aq)} \quad \text{(at pH 1.)}$$

$$VO^+{}_{2(aq)} + 3HA^{+\,Cl^-}{}_{(o)} \rightleftharpoons VO_2Cl(HA)_{3(o)} \text{(in 4·5 HCl)}$$

Solutions of both complexes follow Beer's law and the molar extinction coefficients are 4·15 × 10³ (at 478 nm) and 5·10 × 10³ (at 615 nm). The determination of vanadium at both wavelengths is simple, fast and selective; many cations and anions do not interfere.

HA (in chloroform) has also been used as an extractant for iron(III) in HCl, HNO_3 and H_2SO_4 solutions and the metal determined spectrophotometrically[87] both in aqueous and organic phase. Quantitative extraction of iron(III) takes place when the acid concentration IN. In the aqueous phase iron(III) and HA form three different complexes, depending on the initial iron: HA concentration ratio and pH of the solution. The complexes correspond to FeA^{2+} (violet), FeA_2^+ (orange-red) and FeA_3 (orange-yellow). The complex which is extracted in the organic phase is identical with FeA_3 for which the following mechanism seems to be valid:

$$Fe^{3+}_{(aq)} + 3HA_0 \rightleftharpoons FeA_{3(o)} + 3HA^+_{(aq)}$$

The effect at large number of foreign ions has been studied in determination of iron(III) through formation of FeA_3 in either aqueous phase (λ_{max} 443 nm; ϵ 7·00 × 10^3) or organic phase (λ_{max} 432 nm; ϵ 7·45 × 10^3). The interference of some ions is more pronounced when the determination is made in aqueous phase than when iron is previously extracted into $CHCl_3$ owing to the high selectivity of HA. For example Ba, Cd, Al, Bi, Cr, Co and Ni interfere in the aqueous phase but not in the organic phase because they are not extracted with HA.

Extraction of thorium(IV) and protactinium(V) from HCl solutions by HA (in chloroform) has been investigated and the two elements separated based on their extraction characteristics[88]. Pa can be completely extracted from 0·1–9 N HCl solutions while Th remains in aqueous phase. With increasing concentration of Th in the solution, the percentage of Pa in the extract slightly decreases. Th (in 0·1 N HCl) can only be extracted in presence of chloride ions in accordance with the following mechanism:

$$Th^{4+}_{(aq)} + Cl^-_{(aq)} + 4\,HA_{(o)} \rightleftharpoons Th\,ClA_3.\ HA_{(o)} + 3H$$

The complexes in organic phase can be back extracted with HF or $H_2C_2O_4$ solution (Pa-complex) and HNO_3 (Th-complex).

From 10^{-2} N acid (HCl, HNO_3 or $HClO_4$) solution Ga(III) can be quantitatively extracted[89] with HA (in chloroform) in accordance with:

$$Ga^{3+}_{(aq)} + 3HA_{(o)} \rightleftharpoons GaA_{3(o)} + 3H^+_{(aq)}.$$

With increasing acid concentration, the extraction of Ga(III) diminishes and at 3N acid it remains completely in aqueous phase. Zn(II), present in these acid solutions, remains unextracted and thus can be separated from Ga(III).

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1. Mosher, H. S., *Heterocyclic Compounds*, Ed. R. C. Elderfield, Wiley, New York, 1950.
2. Gautier, J. A. and Renault, J., *Bull. Soc. Chim., France*, 1955, p. 588.
3. Campell, N., *The Chemistry of Carbon Compounds*, Ed. E. H. Rodd, Elsevier, Amsterdam, 1957.
4. Kink, I. C. and Abramo, S. V., *J. Org. Chem.*, 1958, **23**, 1609.
5. Banes, R. A., *The Chemistry of Heterocyclic Compounds*, Ed. A. Weissberger, Vol. 14, Part I, Wiley, New York, 1960.
6. Bader, G. M. and Sasses, W. H. F., *Advances in Heterocyclic Chemistry*, 1963, **2**, 179.
7. Abramovitch, R. A. and Saha, J. G., *Ibid.*, 1966, **6**, 229.
8. Vogel, A. I., *Quantitative Inorganic Analysis*, Longmans, London, 1961.
9. Sandell, E. B., *Colorimetric Determination of Traces of Metals*, New York, 1959.
10. Anderson, R. G. and Nickless, G., *Analyst*, 1967, **92**, 207.
11. Flaschka, H. A. and Barnard, A. J., *Chelates in Analytical Chemistry*, Vol. 4, Marcel Dekker, New York, 1972.
12. Burger, K., *Organic Reagents in Metal Analysis*, Pergamon Press, Oxford, 1972.
13. Kundernatsch, R., *Monatsh.*, 1897, **18**, 613.
14. Von Schickh, O., Binz, A. and Schultz, A., *Ber.*, 1936, **69**, 2593.
15. Plazek, E. and Rodewald, Z., *Roczniki Chem.*, 1936, **16**, 502.
16. Aso, K., *J. Agr. Chem. Soc. Japan*, 1940, **16**, 253; *Chem. Abstr.*, 1940, **34**, 6940.
17. den Hertog, H. J., Schepman, F. R., de Bruyn, J. and Thysse, G. J. E., *Rec. Trav. Chim. Pays-Bas.*, 1950, **69**, 1281; *Chem. Abstr.*, 1951, **45**, 4718 *a*.
18. Sugisawa, H. and Aso, K., *Tohoku J. Agr. Res.*, 1954, **5**, 147; *Chem. Abstr.*, 1956, **50**, 4941 *h*.
19. Clauson-Kaas, N. and Nedenskov, P., *Acta Chim. Scand.*, 1955, **9**, 14.
20. Bain, B. M. and Saxton, J. E., *J. Chem. Soc.*, 1961, p. 5216.
21. den Hertog, H. J. and Bruyn, J. E., *Rec. Trav. Chim. Pays-Bas*, 1951, 70, 182; *Chem. Abstr.*, 1951, **45**, 8013 *d*.
22. Geigy, J. R., A.-G. Fr. 1,477,998 (Cl. C 07 *d*), 21 April 1967, Swiss Appl., 29 April 1965; *Chem. Abstr.*, 1968, **68**, 29606 *s*.
23. —, A.-G. Fr. 1,477,997 (Cl. C 07 *d*), 21 April 1967, Swiss Appl., 29 April 1965; *Chem. Abstr.*, 1968, **68**, 39483 *p*.
24. —, A.-G. Brit., 1,007,036 (Cl. C 07 *d*), Swiss Appl., 29 April 1965; *Chem. Abstr.*, 1968, **68**, 87169 *u*.
25. —, A.-G. Fr. 1,477,996 (Cl. C 07 *d*), 21 April 1967, Swiss Appl., 29 April 1965; *Chem. Abstr.*, 1968, **69**, 27261 *x*.
26. —, A.-G. Brit. 1,123,666 (Cl. C 07 *d*), 14 Aug. 1968, Swiss Appl., 29 April 1965; *Chem. Abstr.*, 1969, 70, 19941 *z*.
27. Aktieselskaket Sadolin and Holmblad, Brit. 765, 529, 9 Jan. 1957; *Chem. Abstr.*, 1957, **51**, 12153.
28. Akiya, S., *Japan J. Exptl. Med.*, 1956, **26**, 91; *Chem. Abstr.*, 1957, **51**, 15699 *h*.

29. Houghton, C., Wright, K. A. and Cain, R. B., *Biochem. J.*, 1968, **106**, 51P.
30. Spinner, E. and White, J. C. B., *J. Chem, Soc.*, 1966, p. 991.
31. — and — *Ibid.*, 1966, p. 996.
32. Curtis, K. E., *Ph.D. Thesis*, University of Waterloo, 1972.
33. — and Atkinson, G. F. *Can. J. Chem.*, 1972, **50**, 1649.
34. Goel, D. P., *Ph.D. Thesis*, University of Delhi, 1969.
35. —, Dutt, Y. and Singh, R. P., *J. Inorg. Nucl. Chem.* 1970, **32**, 3119.
36. Irving, H. and Williams, R. J. P., *Nature*, 1948, **162**, 746.
37. Nasanen, R., *Fisk-For. Finl.*, 1960, **33**, 7.
38. —, *Ibid.*, 1960, **33**, 111.
39. Murakami, Y., Nakamura, K. and Tokunaga, M., *Bull. Chem. Soc. Japan*, 1963, **36**, 6691.
40. Athavale, V. T., Prabhu, L. H. and Vartak, D. G., *J. Inorg. Nucl. Chem.*, 1966, **28**, 1237.
41. Cerkasevic, K. V. and Pluektov, N. S., *Zh. neorg. Khim.*, 1965, **9**, 128.
42. Moeller, T., Martin, D. F., Thompson, L. C., Ferrus, R., Feistel, G. R. and Randall, W. J., *Chem. Rev.*, 1965, **65**, 1.
43. Behrman, E. J. and Goswami, M. N. D., *Anal. Chem.*, 1964, **36**, 2189.
44. Reio, L., *J. Chromatogr.*, 1970, **47**, 60.
45. Adams, R. and Govindachari, T. R., *J. Am. Chem. Soc.*, 1947, **69**, 1806.
46. den Hertog, H. J., Wibaut, J. P., Schepman, F. R. and Van der Wal, A. A., *Rec. Reav. Chim. Pays-Bas.*, 1950, **69**, 700; *Chem. Abstr.*, 1950, **44**, 8920 *b*.
47. Katyal, M., Goel, D. P. and Singh, R. P., *Talanta*, 1968, **15**, 711.
48. Curtis, K. E., Thomson, J. A., Atkinson, G. F., *Anal. Chim. Acta*, 1970, **49**, 351.
49. — and Atkinshn, G. F., Presented at the *Pittsburg Conf. Anal. Chem. Appl. Spectry*, Cleveland, Ohio, 2nd March, 1971.
50. — and — *Anal. Chim Acta*, 1973, *67*, 477.
51 Varga, L. P. and Veatch, F. C., *Anal. Chem.*, 1967, **39**, 1101.
52. Kushwaha, V., Katyal M. and Singh, R. P., *Mikrochim. Acta*, 1972. p. 807.
53. Katyal, M. and Upadhyaya, K. N., *Indian J. Chem. Edu.*, 1974, **4** (2), 5.
54. Mehra, H. C. and Chhatwal, G. R., *Anal. Chim. Acta*, 1974, **72**, 194.
55. Goel, D. P. and Singh, R. P., *Analyst*, 1971, **96**, 123.
56. Kushwaha, V., Katyal, M. and Singh, R. P., *Talanta*, 1974, **21**, 763.
57. Mehta, Y. L., Garg, B. S. and Singh, R. P., *J. Indian Chem. Soc.*, 1976, **53**, 402.
58. —, *Ph.D. Thesis*, University of Delhi, 1975.
59. —, Garg, B. S. and Singh, R. P., *Indian J. Chem.* 1976, **14** A. 1004.
60. —, — and Katyal, M. *Talanta*, 1976, **23**, 71.
61. —, — and — *Anal. Chim. Acta*, 1976, **86**, 323.
62. Sandhu, R. S., Pandeya, K. B. and Singh, R. P., *Indian J. Chem.*, 1976, **14** A, 913.
63. —, *Technology* (in press).
64. Kushwaha, V., *Ph.D. Thesis*, University of Delhi, 1973.
65. Irving, H. M. and Rossotti, H. S., *J. Chem. Soc. B*, 1966, p. 991.
66. — and —, *Ibid.*, 1953, p. 3397.
67. George, P. and McClure, D. S., "Inner orbital splitting" in *Progress in Inorganic Chemistry*, Vol. I, Interscience, New York, 1959, p. 428.
68. Mehta, Y. L., Garg, B. S. and Katyal, M., *J. Chinese Chem. Soc.*, 1974, **21**, 127.
69. —, — and Singh, R. P., *Talanta*, 1976, **23**, 53.
70. Katyal, M., Kushwaha, V. and Singh, R. P., *Analyst*, 1973, **98**, 659.
71. Mehta Y. L., Garg, B. S. and, Singh, R. P., *J. Indian Chem. Soc.* (in press).
72. Gattermann, L. and Skita A., *Ber.*, 1916, **49**, 494.
73. Titov, A. I., *J. Gen. Chem. U.S.S.R.*, 1938, **8**, 1483.
74. McDonald, C. W. and Carter, R., Jr., *Anal. Chem.*, 1969, **41**, 1478.
75. — and Bedenbaugh, J. H., *Mikrochim. Acta*, 1970, p. 612.
76. — and —, *Ibid.*, 1970, p. 474.
77. — and —, *Anal. Chem.*, 1967, **39**, 1476.
78. Kushwaha, V., Singh, R. P. and Katyal, M., *Talanta*, 1973, **20**, 431.
79. Hahn, V. and Kukolja, S., *Croat. Chem. Acta*, 1961, **33**, 137.
80. Blazevic, K. and Hahn, V., *Ibid.*, 1966, **38**, 113.
81. — and Trinajstic, N., *Ibid.*, 1967, **39**, 25.
82. Janko, M. and Herak, M. J., *Ibid.*, 1971, **43**, 179.
83. —, *M.Sc. Thesis*, University of Zagreb, 1968.
84. Herak, M. J., Janko, M. and Blazevic, K., *Croat. Chem. Acta*, 1969, **41**, 85.
85. Janko, M. and Herak, M. J., *Mikrochim Acta*, 1972, p. 198.
86. Tamhina, B. and Herak, M. J., *Ibid.*, 1975, p. 45.
87. Herak, M. J., Janko, M. and Tamhina, B., *Ibid.*, 1973, p. 783.
88. — and —, *J. Inorg. Nucl. Chem.*, 1972, **34**, 2627.
89. Herak, M. J., Tamhina, B. and Jakopcic, K., *Ibid.*, 1973, **35**, 1665.

LETTERS TO THE EDITOR

THE CRYSTAL STRUCTURE OF MONOFUMARATO TETRAAQUO NICKEL(II), DIHYDRATE

THE title compound $[Ni(C_4H_2O_4), 4H_2O], 2H_2O$ was investigated to unravel the scheme of hydrogen bonds in the crystal as also to study the oxygen ligands around the metal.

Crystal data—Monoclinic with $a = 9 \cdot 00$, $b = 10 \cdot 77$, $c = 5 \cdot 14$ Å, $\beta = 98 \cdot 0°$, space group $P2_1/c$, $D_{obs} = 1 \cdot 86$ gm/cm³, $D_{cal} = 1 \cdot 89$ gm/cm³, $Z = 2$. 510 reflexions were collected by Weissenberg photography using single crystals and intensities estimated visually, usual corrections were applied and statistical method used to get the $|F|^2$ values on approximately absolute scale.

Structure determination and comments.—As the space group $P2_1/c$ requires four general formula units in the crystal, both the metal atoms as well as the fumarate anions must occupy centres of symmetry. The three water molecules in the asymmetric unit will occupy general positions. The structure was solved from a three-dimensional Patterson map and refined by successive [001] and [010] Fourier projections. At the present stage of analysis, the R-factors are $R(hko) = 0 \cdot 15$, $R(hol) = 0 \cdot 16$, $R(okl) = 0 \cdot 16$, $R(hkl) = 0 \cdot 18$.

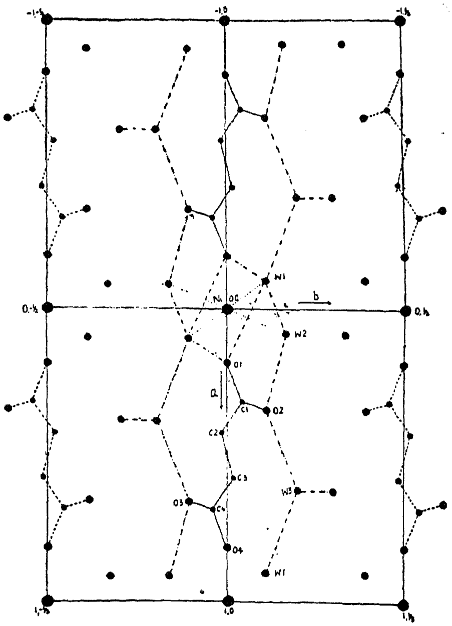

FIG. 1

A view of the crystal structure looking down is shown in Fig. 1 with hydrogen bonds show dashes and dots and metal-oxygen linkages shown by dotted lines.

In the crystal, the geometry of the fum anion is normal. There is six-fold coordi around the metal, Ni – O distances ranging 2·02 to 2·06 Å. The structure is held tog mainly by ionic and hydrogen bond linkage also by van der Waals contacts. Chain fumarate anions are linked *via* water mol parallel to [100]. The hydrogen bonds *via* wat molecules range from 2·63 to 2·91 Å.

As considerable interest lies in locati hydrogen atoms; further work to refine structure is in progress.

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FRACTIONAL CRYSTALLIZATION OF ALUMS—IV

Introduction

WHEN a pair of isomorphous salts is rapidly cry from an aqueous solution, the resulting solid changes in composition continuously. A stud distribution of the salts in the resulting syste considerable theoretical and practical interest.

Abu-Elamayem[1] studied the equilibrium be the solid and liquid phases by the relation

$$Y^2 W(K-1) - Y\{1 + (K-1)(W + a\} \quad K$$

where *a* and Y represent the weight fraction of soluble isomorphous salt in the original mixt crystals separated respectively, W being the fraction of the two salts which are separated a and K is the distribution coefficient.

For nonequilibrium condition, the following e was also proposed:[1,2]

$$-\frac{dY}{dW} = \frac{1}{W}\left[\frac{1}{1 + \frac{1}{K}\left(\frac{1-W}{a-WY} - 1\right)}\right.^{1/m}$$

where *m* is an empirical constant.

In our previously published investigations distribution during crystallization of systems co two inorganic salts, it was confirmed that the ex ments are found to be in agreement with the above two equations.

The object of the present investigation has been to study experimentally the distribution of another pair of isomorphous salts (ammonium alum-potash alum) between aqueous and solid phases and examine the results from the standpoint of equations (1 and 2).

Experimental

Ammonium alum, potash alum and sulphuric acid used were of the B.D.H., AnalaR type. Crystallization was carried out as described before[1]. The mixed crystals were analyzed for total ammonium by alkaline distillation in steam of the ammonia into standard acid and titration of the excess of the latter with standard base.

Results and Discussion

The results of three of the experiments at 0° C are shown in Table I.

TABLE I

Results at 0° *C* — 4 *days* K = 1·5

$a = 0·3333$			$a = 0·5$			$a = 0·6667$		
W	Y	S	W	Y	S	W	Y	S
0·947	0·334	0·92	0·942	0·522	1·15	0·972	0·674	0·80
0·921	0·337	1·35	0·916	0·522	1·66	0·943	0·677	1·35
0·889	0·341	1·93	0·870	0·524	2·46	0·904	0·680	2·02
0·858	0·346	2·50	0·818	0·529	3·40	0·870	0·681	2·64
0·826	0·351	3·11	0·790	0·532	3·93	0·854	0·685	2·92
0·801	0·355	3·60	0·772	0·537	4·26	0·810	0·687	3·75

At 0° C, this system is in a state of non-epuilibrium when the duration of crystallization is only 6 hours. The experimental results are found to fit the theoretical data with K = 1·35. On the other hand, the theoretical data at equilibrium for K = 1·5 were found to agree with the experimental points at 4 days.

When mixed crystals of a definite Y value are needed, the corresponding W value is calculated, then the value of S (weight of solvent) may then be evaluated.

At 20° C, when $a = 0·3333$, the results for 6 hours agree with the theoretical values of K = 1·2 for non-equilibrium. These results are different and lower than those obtained at four days which are found to agree with the theoretical results for K = 1·35 at the equilibrium state.

When $a = 0·5$ or $0·6667$, the similarity of the results at 6 hours and four days is observed. These results are found to agree with the theory and lied on the theoretical values for equilibrium with K = 1·35.

It can be concluded that equilibrium between soild and liquid phases is attained more quickly at 20° C than at 0° C, but it is also affected by the value of a. When a is small, the equilibrium is attained slowly.

The value of Y corresponding to the same value of W and the same value of a are higher at 20° C than that at 0° C. Hence separation of the constituents of a mixture is expected to be more fruitful at higher temperatures.

The equilibrium distribution coefficient D_{eqm} for this system is studied at 20° C by the recrystallization method given previously by Gorstein[4].

The D_{eqm} for potash alum/ammonium alum is 0·737 while D_{eqm}. for ammonium alum/potash alum is 1·356 The system is ideal over the entire range of concentration of components and may be regarded as approximately congruent.

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1. Abu-Elamayem, M. S., *J. Inorg. Nucl. Chem.*, 1964, **26**, 2159.
2. —, Yosri, F., Harfoush, A. and Khalifa, A., *Ibid.*, 1966, **28**, 355.
3. —, Harfoush, A. A. and Salem, S. M., ***Bull. Fac. Sci., Alex. Univ.***, 1976, **8** (To be published).
4. Gorstein, G. I. and Silantyeva, N. I., *J. General Chem.*, 1953, **23**, 1355.

MAGNETIC AN D SPECTRAL STUDIES OF Ni(II), Cu(II) AND Pd(II) CHELATES OF LIGANDS DERIVED FROM 2-HYDROXY-1-NAPTHALDEHYDE AND SOME DIAMINES

RECENTLY Holm *et al.*[1,2], have reviewed the metal chelates of Schiff bases. A perusal of the literature has indicated that no studies have been carried out on the metal chelates of Ni(II), Cu(II) and Pd(II) with N–N′-ethylene or propylene bis-(2-hydroxy-1-napthalidimine) and, the same has now been undertaken.

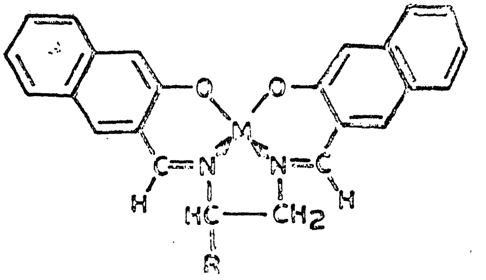
These chelates were prepared by two techniques. (*i*) The preformed Ni(II), Cu(II) or Pd(II) chelates of 2-hydroxy-1-naphthaldehyde were refluxed with an excess of ethylenediamine or propylenediamine and their respective chelates were obtained in which the two $-NH_2$ groups of the diamines condensed with the two aldehydic groups giving rise to tetradentate chelates. (*ii*) By refluxing 2-hydroxy-1-naphthaldehyde with the metal diamines, the chelates were obtained.

The solid chelates were recrystallised from chloroform. These chelates are stable at room temperature but decompose on heating to above 210°. These compounds are insoluble in water but soluble in chloroform and other organic solvents.

All these compounds give satisfactory carbon, hydrogen, nitrogen and metal analyses which suggest 1 : 2 metal-ligand stoichiometry. Magnetic susceptibility measurements were carried out using Gouy apparatus with mercury(II) tetrathiocyanatocobaltate as the reference[3]. The magnetic moment of Cu(II) chelate was found 1·86 B.M. at 303° A whereas Ni(II) and Pd(II) chelates were found diamagnetic.

The chloroform solution of the copper(II) chelate gives one broad band at 570 nm while that of neckel(II) chelate gives two shoulders at 660 nm and 560 nm assignable to the transitions ${}^1A_{1g} \rightarrow {}^1B_{1g}$ and ${}^1A_{1g} \rightarrow {}^1B_{3g}$. Palladium(II) chelate consists of two absorption bands with their peaks at 450 nm and 373 nm which are assigned to the transitions ${}^1A_{1g} \rightarrow {}^1B_{1g}$ and ${}^1A_{1g} \rightarrow {}^1E_{1g}$ respectively. Thus the electronic spectra supported by the magnetic data suggest a square-planar configuration of the metal chelates under investigatiins. This configuration is further supported by the i.r. spectra given below.

The i.r. spectra of these chelates were recorded in nujol mull using sodium chloride prism. The i.r. spectra of the ligand consist of two sharp bands at 3600 and 1640 cm^{-1} which may be due to ν OH and ν C=N, respectively. Two more bands at 1200 and 1030 cm^{-1} due to C–N and C–C stretchings have also been observed. In the case of metal chelates the bands due to ν OH disappeared and the ν C=N frequency was lowered to 1610 cm^{-1} suggesting the co-ordination of azomethine nitrogen to the metal atom. The bands at 670 and 600 cm^{-1} represent metal-oxygen and metal-nitrogen stretchings respectively. Thus the chelates may have the structure as shown in Fig. 1.



Where M = Cu(II), Ni(II) or Pd(III) and R = H or CH_3

FIG. 1. Metal chelates of N–N′ ethylene or propylene bis-(2-hydroxy-1-naphthalidimine).

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---

1. Cotton, F. A., *Prog. Inorg. Chem.*, 1966, **7**, 88.
2. Lyapina, S. S., Mishchenko, V. V., Trailina, E. P., Savich, I. A. and Spitsyn, V. I., *Dokl. Akad. Nauk.*, S.S.S.R., 1970, **190**, 593.
3. Mehta, R. K. and Gupta, R. K., *Indian J. Chem.*, 1973, **11**, 56.

A COLORIMETRIC METHOD FOR THE ESTIMATION OF AMPICILLIN

Introduction

INDIAN Pharmacopoeial and British Pharmacopoeial procedures for the estimation of ampicillin are based upon the conversion of ampicillin to penicillenic acid whose absorbance is measured. British Pharmaceutical Codex procedure for the estimation of ampicillin in syrups and tablets is based on the reaction of hydroxylammonium hydrochloride and ferric chloride with ampicillin both before and after treatment with sodium hydroxide solution. Various analytical methods[1] based on titrimetric, microbiological, uv spectrophotometric, fluorimetric and colorimetric are described by earlier authors. Most of the methoos reported earlier are time consuming.

The α-amino group of ampicillin is reported to play a significant role in its broader antibacterial activity[2]. Keeping this in view an attempt was made to use the amino group for developing a colorimetric method for the estimation of ampicillin. Ampicillin reacts with 4-dimethylaminocinnamaldehyde to form an orange coloured Schiff's base with an absorbance maximum at 480 nm. This forms the basis for the determination of ampicillin by the colorimetric method reported in this paper. The method is simple, accurate and has been successfully utilised for the estimation of ampicillin and its dosage forms.

Experimental

Reagent solution.—Dissolve 0·1 gm of 4-dimethylaminocinnamaldehyde (GR) in 100 ml isopropanol. The reagent can be preserved in a refrigerator for one week.

Standard and Sample Prepatration.—All the samples of ampicillin and its formulations used in the analysis were diluted with a mixture of acetic acid and isopropanol (1 : 1 :5) to give a concentration of 1 mg/ml.

Assay.—Pipette out 1 ml aliquots each of the standard and sample preparations into separate 25 ml standard flasks and make upto the mark with isopropanol. Transfer one ml of these solutions to 10 ml standard flasks, add 1 ml reagent followed by 1 ml acetic acid. Keep the flasks in the boiling water-bath for 2 minutes, allow to cool and dilute to the mark with isopropanol. Measure the absorbance at 480 nm within 15 minutes against reagent blank.

Results and Discussion

Ampicillin was found to react with 4-dimethylaminocinnamaldehyde to produce a Schiff's base. This aldehyde was preferred as a chromogenic agent over vanillin and 4-dimethylaminobenzaldehyde, as deeper colour was obtained. After several experiments isopropanol and glacial acetic acid were found to be the most suitable solvent and acid respectively for optimum colour development. To arrive at an absorbance with less photometric error the reagent requirement was fixed as 1 ml of 0·1% reagent for the range 0·5–12·5 μg of ampicillin per ml.

The colour developed was stable for 15 minutes. The orange coloured chromogen had an absorbance maximum at 480 nm. The colour obeys Beer's law in the concentration range 0·5–12·5 μg per ml of ampicillin. A standard was prepared simultaneously with each experiment to minimise the error.

Several samples of ampicillin, its tablet, capsule, injection and syrup were analysed by the present method and the official methods. The values obtained by the proposed method differ from those of the official methods by a maximum of ± 0·6%, whereas the results obtained by earlier colorimetric method[3] for ampicillin differed by ± 2·0%.

The propsed method is accurate and is suitable for a quick analytical control of ampicillin and its dosage forms.

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1. Klaus Florey, *Analytical Profiles of Drug Substances*, Academic Press, New York and London, 1973, **2**, 1.
2. Wilson, C. O., Gisvold, O. and Doerge, R. F., *Text Book of Organic Medicinal and Pharmaceutical Chemistry*, Pitman Medical Publishing Co. Ltd., London, Fifth Edition, 1966, p. 331.
3. Chowdhury, C., *Indian Journ. of Pharm.*, 1976, **38**, 124.

MERCURIC NITRATE AS A SIMPLE AND NEW CHROMOGENIC SPRAY REAGENT FOR THE DETECTION OF OPIUM AND ITS ALKALOIDS

VARIOUS chromogenic spray reagents for the detection of opium and its alkaloids on thin layer plates have been described in literature[1-4], the most widely used being the Iodoplatinate reagent and Dragendorff's reagent. The iodoplatinate reagent though effective and widely used involves the use of costly platinic chloride. The Dragendorff reagent does not give clear differentiation of the spot from background when the alkaloid is present in low quantities. During the present investigations, mercuric nitrate has proved to be a sensitive chromogenic spray reagent for the detection of opium alkaloids on t.l.c. plates.

Glass plates of size 20 × 20 cm were coated with silica Gel G (E/M) to a thickness of 250 μm and the plates after air drying, were activated in an air oven at 110° for one hour. Then plates were cooled in a desiccator. Methanolic solution of morphine and chloroform solution of codeine, narcotine, papaverine and thebaine were spotted on the plate, and the plate developed in a solvent mixture of benzene: methanol (8 : 2). After the solvent front had moved to a distance of 10–12 cm, the plate was taken out and dried and sprayed with 4% solution of mercuric nitrate in 3% dil HNO_3 and the plates were heated at 110° for 15 minutes.

Results and Discussion

Reddish-brown to yellow spots appeared after heating the plates. Morphine, thebaine, papaverine and narcotine could be detected even at 2 μg level but the minimum detection limit for codeine is 10 μg. The colours of the spots are given in Table I. Hg^{2+} is a well-known electron acceptor[5]. The alkaloids having a lone pair of electrons on the nitrogen atom function as electron

TABLE I

Detection of some opium alkaloids on TLC plates with aqueous mercuric nitrate spray

Alkaloid	Colour	Minimum detectable limit (μg)
Morphine	Reddish brown	2
Codeine	Yellow	10
Thebaine	Yellowish brown	2
Papaverine	Yellow	2
Narcotine	Yellow	2

donors and the formation of charge transfer complexes may be the very likely mechanism of colour formation The reagent was applied for visualizing the alkaloids of opium in crude extracts as well as many other samples of forensic interest.

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1. Santavy, F., Stahl, E. (Editor), *Thin Layer Chromatography*, A Laboratory Hand-book, Springer, New York, 1969.
2. Rama Rao, N. V. and Murthy, H. R. K., *J. Indian Acad. For Sci.*, 1974, **13**, p. 18.
3. Guven, K. C. and Aran, B., *Eczacilik Bull.*, 1973, **15**, 28.
4. Rama Rao, N. V. and Tandon, S. N., *Forensic Science*, 1977 (in Press).
5. Mulliken, R. S. and Person, W. B., in "*Molecular Complexes*—" A lecture and Reprint Volume", Wiley Interscience, 1969, p. 276.

PALLADIUM(II) CHELATES OF SOME *o*-NITROSOPHENOLS

THE literature survey shows that, not much work has been done on the metal complexes of ambidentate ligands containing –OH and –NO groups[1-3]. Here we report the preparation and spectral study of Pd-complexes of the type $Pd(qo)_2$, where qOH represents one of the following:

4-Chloro-, 4-bromo-, 4-methyl-, 5-methoxy-2-nitrosophenols, 3-nitroso-4-hydroxy-coumarin, 2-nitroso-1-naphthol, 1-nitroso-2-naphthol, 3-chloro-, 3-bromo-1-nitroso-2-naphthols.

The complexes of 4-substituted phenols were prepared using the method described by Charalambous *et al.*[1]. Other complexes were prepared in aqueous medium by reacting the metal salt with respective ligands. The pH was adjusted between 2·0 to 4·5. Excess ligand was removed by soxhlet extraction with petroleum ether. The complexes were dried under vacuum over P_2O_5 and analysed. The elemental analysis shows that the ratio of metal to ligand is 1 : 2.

All complexes are non-electrolyte in DMSO. Infrared spectra were recorded on Nujol mull. The main features of spectra are as follows:

(*i*) The band characteristic of intramolecular hydrogen bonded OH which occurs at about 3100 cm^{-1} in the ligand is not observed in spectra of complexes. The characteristic band attributable to co-ordinated water in the region 3400 cm^{-1} is not observed in complexes.

(*ii*) The spectra of complexes show that there is decrease in C=O, C=N and an increase in N–O frequencies; this confirms the formation of complexes. The similarity of spectra of complexes and the ligands where O—H....O bond has been well established enables us to conclude oximic structure in the solid state.

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1. Charalambous, J., Frazer, M. J. and Taylor, F. B., *J. Chem. Soc.*, 1969, 18 A, 2787.
2. Patil, S. V. and Raju, J. R., *Indian J. Chem.*, 1974, 7, 270.
3. Gurrieri, S. and Siracusa, G., *Inorganica Chim. Acta*, 1971, 5, 650.

γ-RADIOLYSIS OF SOME REDOX SYSTEMS

WE had reported recently[1-3] results of our study on certain redox reactions, which though thermodynamically possible do not occur with any detectable rate, but go to completion on gamma irradiation at room temperature.

We present here results of our study on γ radiolysis of aqueous solutions of the following redox mixtures: (*a*) $S_2O_3^{2-}/NO_2^-$, (*b*) $S_2O_3^{2-}/BrO_3^-$, (*c*) $S_2O_3^{2-}/IO_3^-$, (*d*) As(III)/IO_3^-, (*e*) Sb(III)/BrO_3^- and (*f*) Sb(III)/IO_3^- under conditions of neutral pH [(*a*) to (*d*)] and pH = 13 [(*e*) and (*f*)]. The γ-irradiatkons were effected from a ^{60}Co source at a rate of ·8 krad/min, and the actual dose determined by Fricke dosimetry and corrected for respective electron densities in each case. Changes in the concentrations of the reductant $S_2O_3^{2-}$, As(III) and Sb(III) were determined iodometrically and the corresponding G values calculated, for different proportions of initial concentrations of the reductants (0·5 to 50 mM) and the oxidants (1 to 100 mM).

While the radiolysis of a pure thiosulphate solution yields S and H_2S, the same is completely oxidized to $S_4O_6^{2-}$ and SO_4^{2-} in the presence of an oxidant, the G ($-$ $S_2O_3^{2-}$) value being distinctly higher in the presence of a halate ion (3–9) than in the presence of the nitrite ion (0·75 to 1·10).

The G[-As(III)] in the presence of IO_3^- is 3·4 which is much higher than in the case of radiolysis of a pure arsenite solution (2–4).

Similarly in the radiolysis [Sb(III), the G -Sb(III)] is much higher in the presence of an oxidant BrO_3^- or IO_3^-, compared to a pure solution of Sb(III). One noticeable difference between the BrO_3^- and IO_3^- oxidants, is that the G [-Sb (III)] is independent of the BrO_3^- ion concentration whereas it is practically directly proportional to the IO_3^- ion concentration over the same range (0·1 to 0·01 M).

The G values observed in all the cases can be explained on the basis of the participation of the primary radiolysis products of water, *viz.*, e^-_{aq}, H, OH and H_2O_2.

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---

1. Arnikar, H. J., Patil, S. F., Bodhe, A. A. and Pokharkar, R. D., *Radiachem. Radioanal. letters*, 1975, 23 (5–6), 307.
2. — —, Rao, B. S. M., Bodhe, A. A. and Pokharkar, R. D.. *Radiochim. Acta*, 1976, 23, 109.
3. —. —, — and Bedekar, M. J., *Radiochem. Radioanal. Lettres*, 1977, 28 (5–6) 337.

SOLUBILIZATION OF ELASTOIDIN BY ALKALI TREATMENT

ELASTOIDIN found in shark fins is an unusual type of intracellular structural protein belonging to the collagen family[1-3]. The major structural component of elastoidin is considered to be a typical collagen associated with non-collagenous protein rich in tyrosine[4]. They are assumed to be tightly bound and considered to be inseparable except by severe treatment[4,5]. The inherent insolubility of elastoidin fibres renders chemical investigation of its constitution extremely difficult. We now report the solubilization of elastoidin fibres by the action of alkali in saturated salt solution, a treatment known to disrupt intramolecular crosslinkages[6]. Kuhn *et al.*[6] had reported that action of alkali on collagen brings about the splitting of β-components into α-chains by the scission of intramolecular linkages. It is therefore expected that the collagenous and non-collagenous components in elastoidin fibres can be separated by this treatment.

Ealstoidin fibres were exposed to the action of alkali as per the method of Kuhn[6]. The fibres were suspended in 5% sodium hydroxide saturated with sodium sulphate, at 4° C for 15 days. The viscous solution obtained was dialysed against 0·3% acetic acid with several changes and the dialysant was analysed. The analysis of the dialysant showed an increase in the content of hydroxyproline and glycine. Cysteine was absent and tyrosine content is very much reduced (Table I).

TABLE I

Amino acids content of the elastoidin fibre, solubilized elastoidin and shark skin collagen

Sample	Hydroxyproline	Glycine	Tyrosine	Cysteine
	amino acid residues/1000 residues			
1. Elastoidin fibre	55	281	49	2
2. Solubilized elastoidin	58	306	18	0
3. Shark skin collagen*	60	338	3·3	0

* ref. 9.

The results (Table I) show progressive enrichment of collagenous portion in the dialysant. This is corroborated by the determination of the specific rotation of the dialysant which was found to be $(\alpha)_{405} = -978$, the corresponding value for the native collagen is $(\alpha)_{405} = -1020$ and for the denatured collagen $(\alpha)_{405} = -300$[6]. Similarly the denaturation temperature is found to be 31·2° C as compared with the value 29° C for shark skin collagen[7]. The absence of cysteine as well as increase in denaturation temperature may be ascribed either to the loss of cysteine containing peptides during dialysis or to the conversion of these residues to lysinoalanine by β-elimination induced by alkali[8]. The nature of this collagenous component derived from elastoidin is under investigation.

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1. Astbury, W. T., *Trans. Faraday Soc.*, 1938, **34**, 378.
2. McGavin, S., *J. Mol. Biol.*, 1962, **5**, 275.
3. Champetier G. and Faure Fremiet, E., *J. Chem. Phys.*, 1937, **34**, 197.
4. Gross, J. and Dumsha, B., *Biochim. Biophys. Acta*, 1958, **28**, 268.
5. Ramachandran, L. K., *Biochem. Biophys. Research Communs.*, 1962, **6**, 443.
6. Kühn, K., Zimmer, E., Wayhole, P. and Fietzek. P., Hoppe-seylers, '*Zeitschrift. f. Physiol. Chemie*, 1963, **333**, 209.
7. Harrington, W. F. and Von Hippel, P. H., *Advan. Protein Chem.*, 1961, **16**, 76.
8. Bohak, Z., *J. Biol. Chem.*, 1964, **239**, 2878.
9. Piez, K. A., *Science*, 1961, **134**, 841.

POLYPHENOLIC COMPONENTS OF *POLYGONUM CHINENSE* LEAVES

THE genus *Polygonum* (Polygonaceae) comprises more than twenty species. Though there were reports[1] about the medicinal uses of *P. chinense*, no chemical examination of this plant has been reported so far. We present here the results of our chemical investigation of leaves of this plant collected in East Godavari district.

Powdered dried leaves were successively extracted with petroleum ether, ether and ethanol. The petroleum ether extract residue on chromatography yielded only β-sitosterol.

The ether extract on concentration deposited a mixture of flavonoids which was separated on a column of silica gel into kaempferol and quercetin. Their identities were established by direct comparison with authentic specimens (m.m.p., R_f and co-PC).

The ethanolic extract was concentrated to an aq. phase and then successively extracted with ether and ethyl acetate. The ethyl acetate extract on concentration deposited a crystalline compound which was identified as ellagic acid, m.p. > 360°, $C_{14}H_6O_8$ positive Griessmayer's reaction[2], λ_{max} 250, 362 nm, R_f 0·32 in PC (Forestal)[3], tetramethyl ether m.p. 337–38°. The identity was confirmed by direct comparison with synthetic ellagic acid.

The mother liquors of ellagic acid on chromatography over silica gel yielded gallic acid. This was followed by a compound, m.p. > 360°, which gave positive Griessmayer's reaction[2] and had R_f 0·62 (Forestal)[3]. It analysed for $C_{15}H_8O_8$ with one methoxyl and formed tetramethyl ellagic acid on methylation. It had λ_{max} 250, 290 (sh), 365 nm and the addition of sodium acetate caused the splitting of the 250 band to 250 and 264 nm. These properties suggest the identity of the compound as 3–O–methyl ellagic acid[4].

A flavonoid glycoside mixture obtained in the above chromatogram was rechromatographed on silica gel when two glycosides A and B were obtained. Glycoside A, m.p. 228–30°, λ_{max} 267, 367 nm, $C_{21}H_{20}O_{11}$ on hydrolysis yielded kaempferol and glucose. A study of its U.V. spectrum with the addition of $AlCl_3$ and NaOAc suggested that it could be kaempferol-7-O-glucoside[5]. This was confirmed by methylation of the glycoside with diazomethane followed by acid hydrolysis when, besides glucose, 3, 5, 4′-tri-O-methyl kaempferol was obtained and identified by direct comparison with an authentic sample[6].

Glycoside B, m.p. 188–91°, λ_{max} 267, 354 nm, $C_{21}H_{18}O_{12}$ yielded kaempferol and glucuronic acid on acid hydrolysis. Its colour reactions and spectral properties suggested that it could be kaempferol-3-O-glucuronide[7]. This was confirmed by direct chromatographic comparison, kindly carried out by Dr. K. R. Markham, with an authentic sample in H_2O, 15% HOAc and TBA (on cellulose). Its appearance on paper was also identical in U.V. (366 mm), and U.V./NH_3.

To our knowledge this is the first report of the occurrence of 3-O-methyl ellagic acid and kaempferol-3-O-glucuronide in *Polygonum* species and this is the second report of the occurrence of ellagic acid in *Polygonum* species, the first being in *P. bistorta*[8].

Our grateful thanks are due to Dr. K. R. Markham of Chemistry Division, D.S.I.R., Petone, New Zealand, for comparison of kaempferol-3-O-glucuronide, Dr. Piratla N. Rao of Nagarjuna University for identification of the plant material and the U.G.C. for a fellowship (to P.R.S.P.R.).

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1. Chopra, R. N., Nayar, S. L. and Chopra, I. C., *Glossary of Indian Medicinal Plants*, C.S.I.R., New Delhi, 1956, p. 200.
2. Lederer, E., *J. Chem. Soc.*, 1949, p. 2115.
3. Harborne, J. B., *J. Chromatography*, 1959, **2**, 581.
4. Cain, B. F., *New Zealand J. Sci.*, 1963, **6**, 266.
5. Neelakantam, K., Rao, P. S. and Seshadri, T. R., *Proc. Indian Acad. Sci.*, 1943, **17**A, 26.
6. Venkata Rao, D. and Venkata Rao, E., *Planta Medica*, 1972, **22**, 205.
7. Dümkow, K., *Z. Naturforsch.*, 1959, **24** B, 358.
8. Gastirner, F. and Korf, G., *Arch. Pharm.*, 1966, **299**, 640.

CONOPHYTON MISRAI, A NEW STROMATOLITE FORM FROM THE GANGOLIHAT DOLOMITES, KATHPURIA CHHINA AREA, ALMORA DISTRICT, U.P.

A NEW stromatolite form *Conophyton misrai* is recorded from the Gangolihat Dolomites, Kathpuria Chhina area, Almora district, U.P. There are several horizons showing development of this form on the mule track between Kathpuria Chhina and Dhuraphat.

The *Conophyton* has been defined as the stromatolite having conical laminations in which apices point upwards and the axial zone may or may not be present (Donaldson, 1976)[1]. Before the discovery of *Conophyton* from the hot springs of the Yellowstone Park, it was thought to be an extinct group after Precambrian times (Walter *et al.*, 1976)[5]. Now the study of morphogenesis of the *Conophyton* of Yellowstone Park is quite helpful in the better evaluation of this characteristic group in the Precambrian carbonate sequences.

The Gangolihat Dolomites of Middle Riphean age is considered as the youngest formation of the Calc Zone of Pithoragarh, which attains a thickness of about 500 to 700 metres in Almora-Pithoragarh districts, U.P. Valdiya (1969)[4] has, however, considered the entire Calc Zone of Pithoragarh as inverted. Kumar and Tewari (1977)[2] have discussed this aspect. In the present work, the Calc Zone is considered as normal. The stratigraphic succession is given in Table I (after Valdiya, 1969)[4].

TABLE I

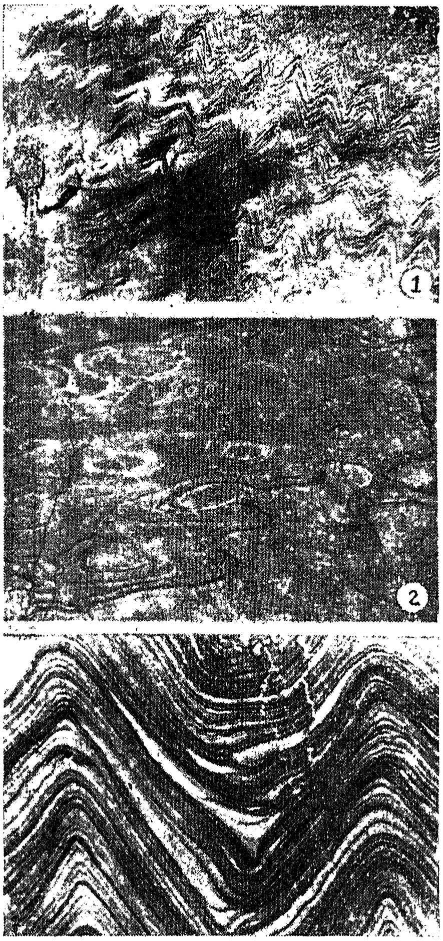
Berinag Quartzites	Orthoquartzites and amphibolites
Calc Zone of Pithoragarh	Gangolihat Dolomites Sor Slates Thalkedar Limestone Rautgara Quartzites
- - - - - - - - - North Almora Thrust - - - - - - - -	
Crystalline Zone of Almora	
- - - - - - - - Schists and gneisses. - - - - - - - -	

Systematic Description

Supergroup	CONOPHYTONIDA	Raaben, 1969
Group	*Conophyton*	Maslov, 1937
Form	*Conophyton misrai*	new form.

(Figs. 1, 2 and 3)

The form is closely packed laterally linked conical layers arranged one over the other. The maximum height is 16 cm. In transverse section, it is circular to elliptical. The maximum diameter is 4 cm. The axial zone is right angle to the bedding plane. The axial angle varies from 70°–80°. In thin section, the dark laminae is continuous and the thickness is in the range of 0·0450 to 0·150 mm. The thickness of light coloured bands is in the range of 0·03 mm to 0·37 mm. The axial zone is not very well marked. The thickness of the dark bands remains constant but there is a general thickening in the light coloured bands in the axial zone. Invariably the laminae of one column is continuous in the adjoining column.



FIGS. 1–3. Fig. 1. Longitudinal Section of *Conophyton misrai*, Kathpuria Chhina area, Almora District, U.P. Fig. 2. Transverse Section of *Conophyton misrai*, same locality as above, × ¼. Fig. 3. Microphotograph of axial zone of *Conophyton misrai*, × 2.

Remarks: The form resembles macrostructurally with *Conophytons* described from the Dismal Lakes Group by Donaldson (1976)[1]. Since he has not described the microstructures of the *Conophytons* of Dismal Lakes, it is difficult to compare the present form with them. The present form also resembles with *Conophyton circulus* described by Raaben (1969)[3] but the axial zone is not as well defined as in *Conophyton circulus.*

Entomology: The form is named in honour of Prof. R. C. Misra, Retired Professor of Geology, Lucknow University, Lucknow.

The other stromatolites recorded from the Gangolihat Dolomites of the Kathpuria Chhina area are *Colonella columnaris, Conophyton garganicus* and *Stratifera.*

The laterally linked *Conophyton* has been used for the interpretation of the environment of deposition. The lack of structures indicative of waves, strong currents and of subaerial exposures in association with Dismal Lakes *Conophytons* leads to the suggestion that these stromatolites formed in a subtidal zone (Donaldson, 1976)[1].

The most conspicuous bedding feature associated with *Conophyton misrai* is parallel lamination, indicating absence of strong currents and wave activity. This can be suggested that the *Conophyton misrai* must have formed in the environment in which currents were either absent or were very weak.

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---

1. Donaldson, J. A., *Stromatolites.* Elsevier Scientific Publishing Company, 1976, p. 523.
2. Kumar, S. and Tewari, V. C., *Journ. Geol. Soc., India*, 1977, (Communicated).
3 Raaben, M. E., *Amer. Journ. Scien.*, 1969, **267**, 1.
4. Valdiya, K. S., *Journ. Geol. Soc. India*, 1969, **10**(1), 1.
5. Walter, M. R., Bauld, J. and Brock, T. D., *Stromatolites,* Elsevier Scientific Publishing Company, 1976, p. 273.

EFFECT OF *AZOLLA* ON THE YIELD OF PADDY WITH AND WITHOUT APPLICATION OF N FERTILIZER

Introduction

Azolla pinnata is commonly found in India in ponds, ditches and channels containing stagnant water[1–4]. The nitrogen fixing blue-green alga *Anabaena azollae* is always found as symbiont in the cavities present at the ventral side of dorsal leaves and nitrogen fixed by the alga is available to the plant[1–5]. This unique property of the plant has drawn the attention of the agriculturists for its utilization in agriculture as organic nitrogen fertilizer for the rice crop[6–9].

The experiments conducted at the Central Rice Research Institute by the author have shown that fern could be multiplied throughout the year when fields are kept under water-logging and fertilized with superphosphate regularly[1–4]. The present study deals with the effect of *Azolla* on the yield of paddy with and without the application of ammonium sulphate.

Experimental

The experiments were conducted at C.R.R.I. farm in replicated plots in Kharif and Rabi to observe the effect of *Azolla pinnata* on the yield of high yielding varieties IR–8, Supriya, CR–1005 and Kalinga-2. The *Azolla* was cultivated separately and applied in the plots before planting of seedlings. (The cost of phosphate fertilizer, pesticide and labour to obtain 10–12 tons of *Azolla* is about Rs. 40.) The freshly harvested *Azolla* contained about 95% water and 4–5% N on dry wt. basis. Incorporation of *Azolla* was done manually by burying in the soil after draining the fields and ammonium sulphate 30 and 50 kg N/ha was applied. Basal doses of 20, 40, 60 and 80 kg N/ha were also tried. The seedlings were transplanted with the spacing of 20 × 15 and 15 × 10 cm in Kharif and Rabi respectively.

Results and Discussion

The increase in grain yield of varieties Supriya and IR–8 was 47% and 25% respectively over control with the application of 10 tons/ha of fresh *Azolla*. Similarly, 47% and 29% respectively were the increase in straw yields of these two varieties. The significant difference in the number and weight of panicle/m^2, etc., are given in Table I.

TABLE I

Effect of Azolla *on the yield of varieties IR–8 and Supriya during* Rabi 1977

Treatments	No. of panicles/m^2		Wt. pf panicles in gm/m^2		Grain yield in kg/ha		Straw yield in kg/ha	
	IR–8	Supriya	IR–8	Supriya	IR–8	Supriya	IR–8	Supriya
Control	339	434	745	548	4722	3489	3607	2571
Azolla (10 tonnes fresh wt/ha)	430	545	880	825	5918	5125	4643	3786
% increase	27	26	18	51	25	47	29	47

The observations of experiments conducted in Kharif and Rabi, using varieties CR-1005 (medium duration) and Kalinga-2 (short duration) are given in Tables II and III. The *Azolla* incorporation produced more yield than unincorporated ones in Kharif (Table II) but no difference was observed in experiments conducted in Rabi. This was because *Azolla* left unincorporated also decomposed within a short period which indicated that mixing of *Azolla* in soil was not essential but it must die to provide nutrients for the crop. In fact *Azolla* plants did not remain for a longer period in field when left uncared and started dying due to over

TABLE II

Effect of Azolla *and N fertilizer on the yield of medium duration variety (CR-1005) during* Kharif, 1976

Treatments	Grain yield		Straw yield	
	kg/ha*	% increase over control	kg/ha*	% increase over control
1. Control (no *Azolla*)	4875	...	4925	..
2. Incorporated *Azolla* (12 tons fresh wt/ha)	5316	9	6175	25
3. Incorporated *Azolla* (24 tons fresh wt/ha)	5605	15	6650	35
4. Unincorporated *Azolla* (12 tons fresh wt/ha)	5158	6	5800	18
5. 20 kg N/ha (basal)	5173	6	5900	20
6. 40 kg N/ha (basal)	5483	13	6725	37
7. 60 kg N/ha (basal)	5819	19	6750	37
8. 80 kg N/ha (basal)	6082	25	6800	38
9. 30 kg N/ha + *Azolla* (12 tons fresh wt/ha)	5664	16	6400	30
10. 50 kg N/ha + *Azolla* (12 tons fresh wt/ha)	6363	31	7750	57

* Mean values.
CD (1%) — 203·58 kg/ha.

TABLE III

Effect of Azolla *and N fertilizer on the yield of short duration variety Kalinga-2 during* Rabi 1977

Treatments	Grain yield		Straw yield	
	kg/ha*	% increase over control	kg/ha*	% increase over control
1. Control (no *Azolla*)	1722	—	1325	—
2. Incorporated *Azolla* (10 tons fresh wt/ha)	2423	41	2087	58
3. Incorporated *Azolla* (20 tons fresh wt/ha)	2623	53	2587	95
4. Unincorporated *Azolla* (10 tons fresh wt/ha)	2400	39	2025	53
5. 20 kg N/ha (basal)	2208	28	2100	58
6. 40 kg N/ha (basal)	3187	85	3437	159
7. 60 kg N ha (basal)	3518	104	3737	182
8. 80 kg N/ha (basal)	3894	126	4650	251
9. 30 kg N/ha + *Azolla* (10 tons fresh wt/ha)	3461	101	2837	114
10. 50 kg N/ha + *Azolla* (10 tons fresh wt/ha)	3576	108	3032	129

* Mean values.
C.D. (1%) — 355·78 kg/ha 526 kg/ha.

growth/nutritional deficiency/rise in temperature/non availability of water and pest infestation. The increase in yield due to application of *Azolla* and N fertilizer in Kalinga-2 (Table III) was more in Rabi in comparison with CR-1005 in Kharif where there was a little increase. The response towards the application of 10–12 tons fresh *Azolla*/ha containing about 20–25 kg N was similar to the application of 30 kg N/ha (ammonium sulphate) and increasing the amount of *Azolla* increased the yield (Tables II and III). The application of 30 and 50 kg N/ha through the ammonium sulphate and 10–12 tons of *Azolla* could increase the yield almost similar to the application of 50–60 and 70–80 kg N/ha through the ammonium sulphate. The increase in straw yield also followed the similar trend.

The shortage and high price of N fertilizers as well as the fear of pollution due to their excess use, initiated the research on alternative technology where utilization of synthetic nitrogen fertilizers could be minimized. The *Azolla* is one of them which will help in reducing the requirement of N fertilizers besides providing organic matter, due to its rapid multiplication (doubling time of 2–4 days under ideal conditions) by utilizing atmospheric nitrogen as nitrogen source. One of the reasons of its rapid multiplication is the occurrence of both photosynthesis and nitrogen fixation in the leaves. The multiplication of the fern was found to be rapid in soils of pH 5·8 to 7·5 t highly acidic or highly alkaline soils. The increase in the yield due to the utilization of *Azolla* is reported upto 40% in different observations[9]. The studies conducted at Central Rice Research Institute also showed significant increase in yield of different high yielding varieties of rice[1–4].

The present study gave rise to the following conclusions :

1. Response towards *Azolla* was comparatively better in short duration varieties (Supriya and Kalinga-2) than the medium duration varieties (like IR-8 and CR-1005). The medium duration varieties probably received more biologically fixed nitrogen due to longer submergence of fields.
2. The response towards *Azolla* and N fertilizer was comparatively better in Rabi than Kharif.
3. The effect of *Azolla* applied on N basis was comparable to the effect of the application of chemical N fertilizer.
4. *Azolla* and chemical N fertilizer could be used together for higher benefit.
5. The unincorporated *Azolla* also increased the yield significantly.

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---

1. Singh, P. K., *IL RISO*, 1977, **26**, 125.
2. —, *Indian Farming*, 1977, **27**, 19.
3. —, *Proc. 64th Indian Sci. Congress*, Part III, 1977, p. 117.
4. —, *Ibid.*, Botany Section, In *Symp Microbial Conservaiion of Nitrogen* 1977, p. 41.
5. Ashton, P. J. and Walmsley, R. D., *Endeavour*. 1976, **39**.
6. Saubert, G. G. P., *Ann. Roy. Bot. Gard. Buitenzerg*, 1949, **51**, 177.
7. Tsujimura, K., Ikeda, F. and Takameto, K., *Jour. Sci. Soil Manure (Tokyo)*, 1957, **28**, 275.
8. Thuyet, T. Q. and Tuan, D. T., In 38 *Vietnamese Studies—Agricultural Problems*, 1973, **4**, 119.
9. Moore, A. W., *Bot. Rev.*, 1969, **35**, 17.

OCCURRENCE OF A COMPLEX VIRAL INFECTION ON *ALTHAEA ROSEA* L.

SYMPTOMS produced by whitefly-transmitted agents have been categorized into mosaic, leaf curl and yellows types[1]. For each of the symptoms different agents are involved. Since, occasionally whiteflies are known to carry more than one virus at a time[2], a disease transmitted by them could not be interpreted as to be caused by a single agent. Cotton leaf curl in Africa, described for Egyptian cotton (*Gossypium barbadense*), was reported to induce yellow mosaic symptoms when transmitted to *G. hirsutum* varieties[3]. Later work[4] indicated the presence of mixed infection with the two whitefly-transmitted diseases.

During the year 1975, many of the hollyhock plants grown at different places within National Botanic Gardens, Lucknow, showed severe vein yellowing symptoms accompanied with leaf curling and dark green vein thickening which rarely bursts into leafy enations. Deformities were easily observed on the undersurface of the leaves which included cupping of the leaves, thickening and zig-zag twisting of the veins. Severe vein yellowing could, however, be observed both on the upper and lower surfaces of the leaf.

An observation of the infected and healthy plants throughout the season, till flowering, showed that the plants, which developed only vein yellowing, grew to a smaller height than the normal with a slight reduction in leaf lamina and produced comparatively faded flowers. The plants showing cupping, leaf curling and thickening of the veins were considerably reduced in size and produced none or only a few small flowers as compared to healthy plants. Severity of the disease in plants, showing both types of symptoms, was enhanced resulting in extreme dwarfing, cupping of the leaves and zig-zag twisting of the swollen veins. The plants neither grew to a size more than 10 inches in height nor produced any flowers. However, the severity of symptoms was completely dependant on the time of infection of each virus at different stages of the plant growth.

Since whitefliesareable to carry more than one virus at a time, whiteflies collected from field-grown *Nicotiana tabacum* plants were multiplied on a series of plant species consisting of *Nicotiana tabacum*, *Phaseolus vulgaris*, *Nicotiana rustica*, *Glycine max*, *Phaseolus aureus*, *Lycopersicon esculentum*, *Zinnia elegans*, *Althaea rosea*, and *Clitoria ternatea*. Transfer of generations of whiteflies to such a series of host plants made them completely virus-free. None of the tests done with whiteflies grown on *Clitoria ternatea* produced any symptoms on the aforesaid test plants. Non-viruliferous whiteflies thus multiplied were allowed to feed on diseased plants of hollyhock showing both leaf curl and vein yellowing symptoms. The handling of the whiteflies during transmission test was carried out using a method[5] described earlier. Viruliferous whiteflies were transferred at a rate of 20 per test plant. In each test, at least five plants of one species were used.

Data included in Table I showed that only 2 plant species showed vein yellowing symptoms while five others produced only leaf curl accompanied by vein thickening. *Althaea rosea*, however, developed both vein yellowing and leaf curl symptoms. Back inoculation tests from infected plants to *Althaea rosea* produced either only vein yellowing or leaf curl and vein thickening type of symptoms (Fig. 1). It is, therefore, inferred that *Abelmoschus esculentus* can be used to separate vein yellowing agent while several species of Solanaceae mentioned in Table I can be a tool for separating leaf curl agent from the doubly infected plants.

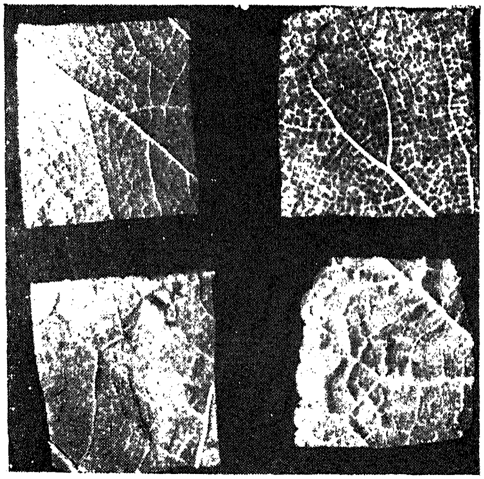

FIG. 1. View of leaf portions of *Althaea rosea*.
Top left = Healthy
Top right = Vein yellowing only.
Bottom left = Vein yellowing + enations
Bottom right = Swollen veins showing yellowing accompanied with leaf cupping.

TABLE I

Transmission of a complex infection of Althaea rosea *to other hosts through whiteflies*

Hosts	Symptoms	Incubation period in host	Back Inoculation on *Althaea rosea*
Ageratum conyzoides	..	NA	..
Abelmoschus esculentus	VY	18–22 Days	VY
A. moschatus	VY	20–25 Days	VY
Althaea rosea	LC, VY, VT	25–30 Days	LC, VT, VY
Capsicum annuum	LC, VT	15–20 Days	LC, VT
Carica papaya	LC, VT	25–30 Days	—
Cucumis sativus	—	NA	—
Dolichos lablab	—	NA	—
Lycopersicum esculentum	LC, VT	15–20 Days	LC, VT
Nicotiana rustica	LC, VT	18–22 Days	LC, VT
N. tabacum	LC, VT	20–22 Days	LC, VT

— = No symptoms,
LC = Leaf Curling,
NA = Not applicable,
VT = Vein thickening,
VY = Vein yellowing.

Susceptibility of the different hosts tested, indicates the involvement of hibiscus vein yellowing[6] and tobacco leaf curl[7] viruses in complex infection of *Althae rosea* plants. This is being reported for the first time along with the separation of individual virus from the mixture using hosts as filter.

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---

1. Costa, A. S., *Ann. Rev. Phytopathol.*, 1976, **14**, 429.

2. Varma, P. M., *Natl. Inst. Sci. India Bull.*, 1963, **24**, 11.
3. Kirkpatrick, T. W., *Bull. Entcmol. Res.*, 1931, **22**, 323.
4. Hutchinson, J. B., Knight, R. L. and Pearson, E. O., *J. Gen. Virol.*, 1950, 20, 217.
5. Verma, H. N., Srivastava, K. M. and Mathur. A. K., *Plt. Dis. Reptr.*, 1975, **59**, 494.
6. Capoor, S. P. and Varma, P. M., *Indian J. Agric. Sci.*, 1950, **20**, 217.
7. Smith, K. M., *A Textbook of Plant Virus Diseases* Longman Group Ltd., London, 1972, p. 684.

FREE PROLINE ACCUMULATION AND REDUCTION IN RWC UNDER MOISTURE STRESS IN GENOTYPES OF SAFFLOWER

SCREENING of varieties for relative drought tolerance is of practical significance in that it helps in recommending the variety directly for cultivation under rainfed conditions. Several laboratory tests have been developed and used. A few studies show a correlation between performance in laboratory tests and actual field trial. Accumulation of free proline in leaves appears to be an adaptive mechanism for drought tolerance. A relationship between proline accumulation under stress and yield stability index has been shown in barley genotypes (Singh *et al.*[1], 1972). Such a relationship has been established (Mehkri *et al.*[2], 1976) in groundnut genotypes also, Decrease in relative water content (RWC) in the leaf under a given stress is another parameter (Barrs[3], 1968). Using these two parameters, differences in safflower genotypes were studied as a measure of drought tolerance.

Six genotypes of safflower SF–11, 143–20, S–2, C–438, A–1 and 16–4–P_2–2 were grown in a single pot (18 × 12 × 12″ size). One month old seedlings were subjected to stress by withholding water for 5 days. Determinations of proline and RWC were done on 4th and 5th day after withholding water and continued up to three days after rewatering. Rewatering was done after 5th day. Since the genotypes were raised in the same pot and subjected to stress, the attributes on proline accumulation and RWC are directly comparable between genotypes.

Free proline in the leaves was determined using the method of Bates *et al.*[4] (1973), and RWC by the method of Barrs and Weatherly[5] (1976).

The data in Table 1 indicated that free proline increased in the leaves of all genotypes under stress. But the magnitude of the increase differed among genotypes. The variety 143–20 showed a 35·9 fold increase in proline over the control followed by the variety S–2. The varieties A–1 and 16-4-P_2-2 showed relatively lesser increase in proline under stress. However, the absolute amounts of proline when compared among varieties indicated that SF–11 had the highest under peak stress followed by 143–20. Varieties A-1 and 16–4–P_2–2 were still low in the absolute amount also. On rewatering the decrease in proline was faster in SF–11 and 143–20 compared to other varieties (Fig. 1). This aspect could be a measure of the resumption of the metabolic activity following rewatering. Under conditions of intermittent moisture stress this point is of importance. Differences amongst genotypes in free proline accumulation have been noticed by other workers also in sorghum (Sinha and Rajagopal[6], 1975).

The data on the reduction of RWC under stress bear a significant relationship with the ability to accumulate proline. The varieties 143–20 and S–2 which showed

TABLE I

Variety	Proline			RWC		
	Control	Stress 120 hr after	No. of times increase over Control	Control	Stress 120 hr after	% reduction on stress
SF–11	102	2620	25·7	86·5	42·7	50·6
143–20	68	2445	35·9	84·3	45·8	45·6
S–2	70	2260	32·0	85·0	43·1	49·3
C–438	82	2015	24·5	90·2	41·4	54·1
A–1	81	1645	20·3	87·4	42·0	51·9
16–4–P_2–2	58	1307	22·5	88·0	42·7	51·5

Free proline (g/gm fresh wt.) and RWC in genotypes of safflower 120 hours after withholding water. All the genotypes were raised in the same pot. Control values represent data collected before withholding water.

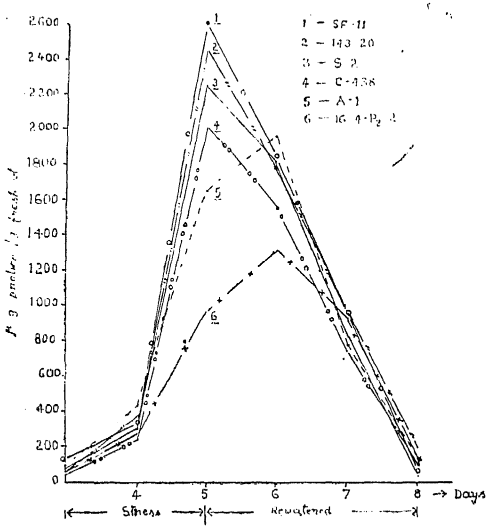

FIG. I

a high magnitude of proline accumulation under stress also showed less reduction in RWC. This relationship in other varieties was not consistent. The main attribute for better drought tolerance of sorghum compared to maize is its higher RWC (Sanchez Diaz and Kramer[7], 1971). In this group of varieties tested, genotypes 143–20 and S–2 are relatively more tolerant to moisture stress than the other genotypes.

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---

1. Singh, T. N., Aspinall, D. and Paleg, L. G., *Nature*, 1972, **236**, 188.
2. Mehkri, A. A., Shashidhar, V.R., Udaya Kumar, M. and Krishna Sastry, K. S., *Indian J. Plant Physiol.*, 1976 (In press).
3. Barrs, H. D., *Water Deficits and Plant Growth*, Vol. I, T. T. Koslowski (ed.), Academic Press, New York and London, 1968, p. 235.
4. Bates, L. S., Waldren, R. P. and Teare I. D., *Plant and Soils*, 1973, p. 38.
5. Barrs, H. D. and Weatherley, P. R., *Aust. J. Biol. Sci.*, 1962, **15**, 413.
6. Sinha, S. K. and Rajagopal, V., *Proc. Symposium on "Crop Plant Response to Environmental Stress"*. IARI, New Delhi, 1975.
7. Sanchez Diaz, M. S. and Kramer, P. J., *Plant Physiol.*, 1971, **48**, 613.

INFLUENCE OF RADISH MOSAIC VIRUS INFECTION ON ASCORBIC ACID CONTENT OF RADISH (*RAPHANUS SATIVUS* L.)

DURING the survey of virus diseases of crop plants in Gorakhpur, radish plants were found severely infected with radish mosaic virus, identified in this laboratory. The present investigation was undertaken to study the effect of radish mosaic virus infection on ascorbic acid content of leaves and roots of radish.

Radish was grown in pots filled with sterilized soil and kept in insect-proof glass-house. One lot of 25 plants was inoculated with the virus and the other lot of 25 plants served as control. Observations were made at 0, 10, 20, 30 and 40 days after inoculation. The ascorbic acid content was determined by the method of Ghosh *et al.*[1]. Results are given in Table I. Data are based on the average of three replications. All the results are significant at 5% level.

TABLE I

Ascorbic acid content (mg/g fresh wt.) of radish leaves and roots infected with radish mosaic virus

Days after inoculation	Leaf		Root	
	Healthy	Diseased	Healthy	Diseased
0	0·25	0·25	0·05	0·05
10	0·30	0·27	0·07	0·065
20	0·42	0·36	0·10	0·09
30	0·60	0·46	0·12	0·10
40	0·82	0·65	0·17	0·15

It is evident from the data given in Table I, that both in healthy and diseased leaves and root samples the ascorbic acid content gradually increases with the age of plant but in the diseased samples it is always less than the healthy counterparts. The maximum decrease in leaves is 23% and in roots it is 16·6% at 30 days after inoculation.

The decrease in ascorbic acid contents due to virus infection has also been reported by Moycho and Niemyski[2]. Diener[3] observed increased respiratory rate in virus infected plants. It is possible that due to high rate of respiration in diseased leaves and roots, the ascorbic acid is oxidised at a higher rate which caused decrease in the ascorbic acid at 10, 20, 30 and 40 days after inoculation. After 30 days of inoculation the symptoms on the plant were most severe and pronounced with maximum concentration of virus, thus there was a maximum decrease. Similar results have also been observed by Joyshi *et al.*[4] in cape gooseberry fruits (ripe and unripe) infected with cucumber mosaic virus.

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1. Ghosh, A. K., Bhargava, S. N. and Tandon, R. N., *Indian Phytopath.*, 1966, **19**, 262.
2. Moycho, W. and Niemyski, K., *Acta Agrobot.*, 1962, **12**, 261.
3. Diener, T. O., *Ann. Rev. Phytopath.*, 1963, **1**, 197.
4. Joshi, R. D., Dubey, L. N. and Gupta, A. K., *Sci. and Cult.*, 1977, **43**, 40.

A MUSCARDINE DISEASE OF TOBACCO LEAF-EATING CATERPILLAR

THE leaf-eating caterpillar *Spodoptera litura* on tobacco is an important pest in India. During our investigations on biological control of insect pests of agricultural crops utilizing fungi, a species of *Nomuraea* Maubl. (Hyphomycetes) was repeatedly isolated in pure culture from the caterpillars of *Spodoptera litura* at Poona during the rainy season of 1976. The fungus made slow growth on potato dextrose agar at 25°–28° C producing a colony 5 mm in diam. at the end of 10 days. Colonies were convex, shining, amorphous, initially white, later turning light-green with profuse sporulation.

Pathogenicity trials were conducted by spraying a dense aqueous spore suspension of the fungus on healthy caterpillars (7 days old). The fungus proved to be a virulent pathogen inflicting 100% mortality of the inoculated caterpillars after 4 to 6 days (Fig. 1). The fungus was reisolated from such infected caterpillars, satisfying the well-known Koch's postulates.

The fungus showed the following morphological characters :

Mycelium sub-hyaline, septate and highly branched. Conidiophores erect, septate, arising from the submerged hyphae, upto 240 μm in length, and 2·5–4 μm in width, bearing whorls of short branches originating just below the septum. These branches then gave rise to whorls of 2–3 phialides. Branches were short, cylindrical, measuring 5–7·8 × 2·1–3·5 μm, conidia dry, catenate, broadly ellipsoidal to cylindrical, smooth, pale green, phialidic, blastic, 2·5–4 × 1·8–2·6 μm (Fig. 2).

Based on detailed morphological characters, the fungus was identified as *Nomuraea rileyi* (Farlow) Samson[1]. This constitutes the first known report of this entomogenous fungus on *Spodoptera litura* from India[2].

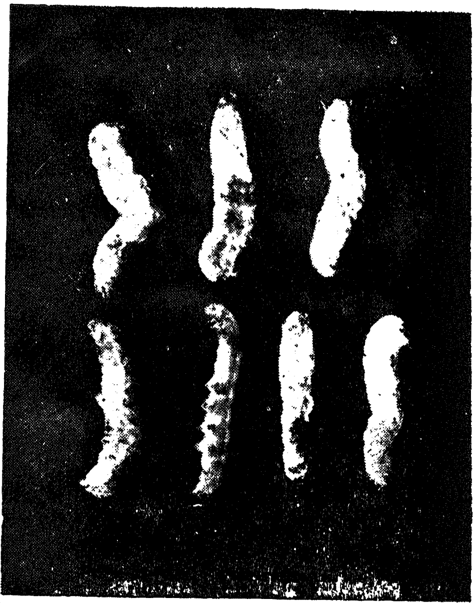

FIG. 1. Caterpillars showing infection due to *Nomuraea rileyi* (Farlow) Samson.

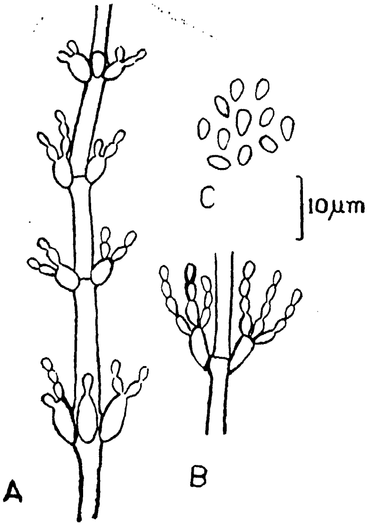

FIG. 2. Morphology of *Nomuraea rileyi* (Farlow) Samson. A, A fertile branch ; B, A part of conidiophore with short branches bearing phialides and catenate conidia ; C, Conidia.

The material has been deposited at M.A.C.S., Poona, under A.M.H. No. 3366 and also at C.M.I. Kew, England, under No. I.M.I. 210318.

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1. Kish, L. P., Samson, R. A. and Allen, G. E., *J. Invert. Path.*, 1974, 24, 154.
2. Mukerji, K. G. and Juneja, R. C., *Fungi of India* (1962–1972), Emkey Publ., Delhi, 1975, p. 224.

EFFECT OF ALAR, CCC AND ETHREL ON IAA-OXIDASE ACTIVITY OF LAMINA OF THE BANANA CV. MONTHAN

CONTROL of plant growth in horticultural crops particularly in fruit trees has received the attention of research workers and fruit growers alike for several decades. One of the most recent developments is the use of Alar. CCC and Ethrel to control plant size. They regulate the physiology of treated plants. The effects of CCC and Alar on plants are opposite to those of gibberellin[1–3]. But Stuart and Cathey[4] felt that growth retardants should not be called as anti-gibberellins. Many workers have reported that the level of auxin was lowered following treatment with growth retardants. Kuraishi and Muir[5] and Knypl[6] found that CCC retarded or inhibited auxin mediated growth mechanism. They also concluded that these compounds do not interact directly with gibberellin but with auxin. Thus the terminology has caused controversy. In the auxin studies the possibility of ethylene interference had been recognised when Van der Laan[7] demonstrated that exposure to ethylene would suppress the amounts of diffusible auxin, presumably by an increase in the activity of IAA-oxidase[8]. Auxin effects are often opposite to those of ethylene[9]. The relevance of these findings in plants is not clear. With this in view experiments were conducted to study the effect of Alar, CCC and Ethrel on the IAA-oxidase activity of the lamina of the banana CV. Monthan.

The tall growing culinary bana CV. Monthan was selected for the study. When the plants were two months old, foliar sprays of Ethrel (2, Chloroethyl phosphonic acid) at 250 and 500 ppm, CCC (2-chloroethyl trimethyl ammonium chloride) at 1000 and 2000 ppm and Alar-85 (Succinic acid-2, 2-dimethyl hydrazide) at 250 and 500 ppm were given till run off. After spraying, leaf samples (third leaf from the apex as per Hewitt[10] and Murray[11]) were drawn at 3-day intervals. The middle lamina portion of the third leaf from the apex were chosen for analysis. The colorimetric method[12] was adopted to estimate the IAA-oxidase activity. The oxidation of IAA was measured in Spectronic-20 at 540 mμ. Optical densities were converted to μg of IAA, employing a standard curve. Activity was expressed as μg IAA destroyed per gram of fresh tissue in two hours.

It could be seen from Table I that Ethrel 250 ppm increased the IAA-oxidase activity, except on the 3rd day. On the 9th day after spray, a maximum of 38·32% activity was recorded at 500 ppm of Ethrel. The increased activity of IAA-Oxidase would have thus caused decrease in auxin level leading to reduced vegetative growth of banana[13]. This lends support to the reports of Abeles[9] and Burg and Burg[14] that ethylene causes decrease in the auxin level.

TABLE I

Effect of growth regulators on IAA-Oxidase activity of Lamina of the CV. Monthan Banana (μg IAA Destroyed/ G fresh tissue/2 hours)

Treatments	ppm	Days after spraying				
		3	6	9	12	15
Ethrel	250	529	654	738	679	797
	500	582	642	888	685	817
CCC	1000	608	667	413	334	771
	2000	638	608	396	433	796
Alar	250	417	708	429	675	750
	500	567	650	679	629	792
Control		563	617	642	658	717

Alar at 500 ppm also showed increase in IAA-oxidase activity except on the 12th day after spray and at 250 ppm it increased the activity from 6 to 15th day. Thus Alar also should have suppressed the endogenous auxin level, supporting the views of Halevy[15] and Kuraishi and Muir[5]. CCC caused increased IAA-oxidase activity initially, decreased activity during the intermediary stages and finally on 15th day, it again increased the activity. In banana, Annadurai[13] reported slight increase in growth due to CCC sprays. So it is presumed that CCC may likely to interfere in the synthetic pathway of gibberllin, late in the sequence. Such a change would stimulate the growth by CCC, due to switching of the biosynthetic pathway from the production of normal gibberellin to an abnormal (and incidentally more active) homologue[16].

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---

1. Tolbert, N. E., *J. Biol. Chem.*, 1960, **235**, 475.
2. Lockhort, J. A., *Pl. Physiol.*, 1962, 32, 204.
3. Cathey, H. M., *Ann. Rev. Plant Physiol.*, 1964, **15**, 271.
4. Stuart, N. W. and Cathey, H. M.,, *Science*, 1961, **134**, 50.
5. Kuraishi, S. and Muir, R. M., *Plant Physiol.*, 1963, **38**, 19.
6. Knypl, J. S., *Bio Chem. Physiol. Pflanzen.*, 1964, **161**, 1.
7. Van Der Laan, P. A., *Rec. Trav. Bot. Neer.* 1934, **31**, 691.
8. Beyer, E. M. and Morgan, P. W., *Plant Physiol.*, 1970, **46**, 352.
9. Abeles, F. B., *Ann. Rev. Plant Physiol.*, 1972, **23**, 259.
10. Hewitt, C. W., *Emp. J. expt. agric.*, 1955, **23**, 11.
11. Murray, D. B., *Trop. agric.*, 1960, 37, 92.
12. Parthasarathy, K., Balu, D. R. C. and Rao, P. S., *Proc. Indian Acad. Sci.*, 1970, **72**, 277.
13. Annadurai, S., *M.Sc. (Agric) Thesis*, Tamil Nadu Agricultural University, 1976.
14. Burg, S. P. and Burg, E. A., *Proc. Natl. Acad. Sci.*, 1966, **55**, 262.
15. Halevy, A. H., *Plant Physiol.*, 1963, **38**, 731.
16. Audus, L. J., *Plant Growth Substances*, Leonand Hill, London, 1972.

MOSAIC DISEASE OF NASTURTIUM (*TROPAEOLUM MAJUS* L.)—A NEW RECORD FROM INDIA

MOSAIC disease of Nasturtium has been reported from California (Jensen[1], 1950) and Brazil (Silberschmidt[2], 1953); from India it is the first record. In the year 1976–77, the authors observed this disease in Taj nursery, Agra. Besides the typical mosaic symptoms, the infected Nasturtium plants showed vein clearing and cupping of leaves in both young and mature plants. The infected plants were stunted and excessively branched. The leaves were greatly reduced in size; lamina showed crinckling and lobed margin (Fig. 1. B.). The flowering was either absent or only few.

The virus is readily transmitted from host to host both by graft and sap prepared in phosphate buffer (pH–7·0, 0·02 M) and applied with carborundum powder as an abrasive. Out of the 8 plants (*viz.*, *Chenopodium amaranticolor* Coste and Regn, *C. album* L., *C. murale* L., *Gomphrena globosa* L., *Datura stramonium* L., *Nicotiana glutinosa* L., *N. tobacum* L. var. white burley, *N. tobacum* L. var. xanthi), commonly used as local lesion hosts, the virus, gave chlorotic local lesions only on *Chenopodium amaranticolor* Coste and Regn, (Fig. 1 C) while all the others did not show the presence of the virus when back inoculations were done on both the host as well as *Chenopodium amaranticolor* Coste and Regn.

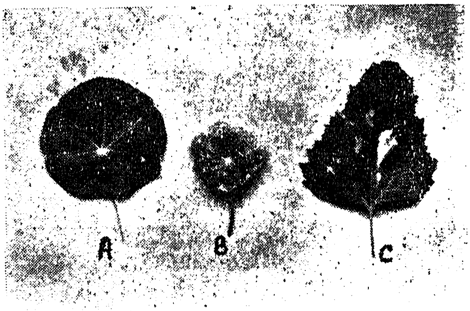

FIG. 1. A, Healthy leaf; B, Diseased leaf; and C, Local lesion on *C, amaranticolor* Coste and Regn.

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1. Jensen, D. D., *Phytopathology* (*Abstr.*), 1950, 40, 967.
2. Silberschmidt, K., *Phytopathology*, 1953, **43**, 304.

STUDIES ON DEGRADATION OF ANTIFUNGAL ANTIBIOTICS

THE use of antibiotics in plant disease control has assumed importance[5,6]. In India, antibiotics have given encouraging results in seed treatment against seed-borne diseases[1–4]. Although antibiotics have been evaluated for seed disinfection, no information is available about their behaviour when treated seeds are stored. Studies on this aspect are reported.

Paddy seeds were treated with piomy and aureofungin at 500 ppm. The biological activity of the antibitotics soon after seed treatment was determined by plating the seeds on potato-dextose-agar seeded with *Helminthosporium oryzae*. The inhibition zone was observed around the treated seeds indicating the presence of antibiotics. The treated and untreated seeds were stored in cloth bags at room temperature, for a period of one year. After this period the activity was reassessed by the same method. In all, three experiments were carried out, and the data based on these studies are presented in Table I.

TABLE I

Effect of seed treatment with antibiotics against H. oryzae *and seed germination*

Treatment	Efficacy soon after seed treatment	Per cent seed germination	Efficacy after one year seed storage	Per cent seed germination
Piomy	+	90	..	88
Aureofungin	+	91	..	85
Check		88		76

+ denotes effective   — denotes ineffective

It is evident from Table I, that piomy and aureofungin are rendered biologically inactive on the seed after one year, suggesting their degradation in storage and consequent loss of fungicidal properties.

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---

1. Dharam Vir and Raychaudhuri, S. P., *Hindustan Antibiot. Bull.*, 1968, **10**, 319.
2. — and —, *Ibid.*, 1969, **11**, 166.
3. — and —, *Plant Protec. Bull.*, 1971, **22**, 1.
4. Padmanabhan, S. Y., *Indian Phytopath.*, 1974, **27**, 1.
5. Raychaudhuri, S. P. and Dharam Vir., *Proc. 1st Intersec. Congress of International Association of Microbiological Societies*, Vol. III (Ed. Hasegawa, H.), Science Council of Japan, Tokyo, 1975, p. 598.
6. Thirumalachar, M. J., *Adv. Appl. Microbiol.*, 1968, **10**, 313.

TOPOGRAPHICAL RELATIONSHIP BETWEEN THE BLASTOCYST AND THE UTERUS IN THE RHINOLOPHID BAT, *RHINOLOPHUS ROUXI* (TEMMINCK)

THE mammalian blastocyst bears a specific topographical relationship with the morphology of the uterus at the time of implantation, and this is usually constant within the family and sometimes within the order (Mossman[1,2,3]). Among the bats, the orientation of the embryonic mass of the implanting blastocyst is mesometrial in Megachiroptera (Keibel[4], Moghe[5], Mossman[1]), and antimesometrial in the Microchiroptera (Daval[6], Ramaswami[7], Wimsatt[8], Gopalakrishna[9]). Most of the earlier work on the embryology of Microchiroptera relates to members of the family Vespertilionidae. During the past 20 years the embryology of members of several families of Microchiroptera has revealed that the orientation of the embryonic mass differs not only in different families of Microchiroptera (Gopalakrishna[10,11], Gopalakrishna and Moghe[12]) but also in different individuals of the same species, in a few cases (Gopalakrishna and Khaparde[13], Gopalakrishna and Karim[14]). Where the orientation of the embryonic mass has been shown to be variable in the same species, the blastocysts described were at a very early stage of development, and the embryonic mass in all the cases was still spherical. Gopalakrishna and Khaparde[13], and Gopalakrishna and Karim[14] suggested that the embryonic knob in these bats may rotate within the blastocyst, as in the case of the mouse (Kirby, Potts and Wilson[15]).

While making a detailed study of the early development of the Rufous Horse-shoe Bat, *Rhinolophus rouxi*, the author examined eight late implanted bilaminar blastocysts, all nearly at the same stage of development. In every case the blastocyst was in a large implantation chamber of the uterus, and the wall of the blastocyst was in intimate contact with the endometrium since the uterine epithelium was lost from all sides of the implantation chamber. The embryonic area had expanded into a flat disc. Among the eight blastocysts, the embryonic disc was oriented towards the antimesometrial side in two, towards the mesometrial side in one, towards the lateral side in two, towards the medial side in two and between the lateral and mesometrial side in one.

Whereas the earlier descriptions of variable orientation of the embryonic mass referred to early blastocysts having spherical embryonic mass[13,14], the present study refers to advanced blastocysts with expanded embryonic disc. Hence the explanation that the embryonic mass may rotate within the blastocyst covering is not tenable in the present case. The final position of the placental disc in *Rhinolophus rouxi* is however invariably mesometrial (Gopalakrishna and Bhiwgade[16]). In all bats, except *Tadarida braziliensis cynocephala* (Stephens[17]), the final placental disc is invariably formed on the side towards which the embryonic mass is directed at the stage of implantation. In *Tadarida braziliensis cynocephala*[17] the early orientation of the embryonic mass is antimesometrial but the final placenta is formed on the mesometrial side.

Rhinolophus rouxi is evidently an exception among Chiroptera where, regardless of the variable orientation of the embryonic disc in advanced implanted blastocysts, the final placental disc is invariably located on the mesometrial side. Our knowledge of the embryology of bats does not suggest any explanation of this anomaly except to presume that the allantois grows towards the mesometrial side through the exocoelom formed by the separation of the yolk sac splanchnopleaure from the chorion in all the blastocysts except in those in which the embryonic disc is already oriented towards the mesometrial side. In the latter case, the allantois grows as in other bats (except *Tadarida braziliensis cynocephala*[17]) towards the dorsal side of the embryonic disc since the yoll sac lies on the ventral side of the embryonic disc.

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1. Mossman, H. W., *Contributions to Embryology*, Carnegie Institute of Washington, 1937, **26**, 127.
2. —, *Jour. Mammal.*, 1953, **34**, 289.
3. —, In *The Biology of the Blastocyst*, The Univ., Chicago Press, 1971, p. 49.
4. Keibel, F., *Arch. f. Mikr. Anat.*, 1922, **96**, 528.
5. Moghe, M. A., *Proc. Nat. Inst. Sci. India*, 1956, **22**, 49.
6. Duval, M. *Jour. de Anat. et de la Physiol.*, 1894, **31**, 93.
7. Ramaswami, L. S., *Half Yearly Jour. Mysore Univ.*, 1932, **7**, 1.
8. Wimsatt, W. A., *Amer. Jour. Anat.*, 1944, **74**, 355.
9. Gopalakrishna, A., *Proc. Indian Acad. Sci.*, 1947, **26**, 219.
10. —, *Jour. Morphol.*, 1958, **102**, 157.
11. —, *Jour. Zool. Soc. India*, 1971, **23**, 55.
12. — and Moghe, M. A., *Zeitschr. f. Anat. u. Entwickelungsgesch.*, 1960, **122**, 137.
13. — and Khapairde, M. S., *Curr. Sci.*, 1972, **41**, 738.
14. — and Karim, K. B., *Ibid.*, 1973, **42**, 860.
15. Kirby, D. R. S., Potts, D. M. and Wilson, I. B., *Jour. Embryol. Exp. Morphol.*, 1967, **17**, 527.
16. Gopalakrishna, A. and Bhiwgade, D. A., *Curr. Sci.*, 1974, **43**, 516.
17. Stephens, R. J., *Amer. Jour. Anat.*, 1962, **111**, 259.

CHANGES IN TISSUE GLYCOGEN OF A FRESH WATER CATFISH *HETEROPENUSTIS FOSSILIS* (BLOCH) DUE TO MERCURY INTOXICATION

THERE has been much anxiety in many countries due to adverse effects arising from heavy metal intoxication as a result of rapid industrialization. Due to mercury intoxication minamata disease, congenital mental retardation, injury to kidney, suppression of urine and ultimately death from kidney failure, corrosion of gastrointestinal tract, etc., are caused[1]. Though heavy metal intoxication has been studied by numerous investigators,[2–6] the effect of mercury intoxication on glycogen content of fish tissues has not been studied, In the present investigation the effect of various concentrations (5, 25 and 50 ppm) of mercuric nitrate on glycogen content of brain, liver, kidney and muscle of *H. fossilis* has been investigated.

Fifteen live *H. fossils* (18–20 cm) were acclimatized in the laboratory for 3-4 days. A group of 5 fishes was kept in the glass aquaria containing 5, 25 and 50 ppm of mercuric nitrate for 2-3 hours. Their liver, brain, kidney and muscle were homogenised and centrifuged for 20 minutes at 3,000 rpm in 5 ml of 1·5% KOH and processed for glycogen content[7]. Each experiment was repeated 4 times and the data were subjected to statistical analysis. Glycogen content of the above–mentioned tissues of untreated fish was taken as the control.

Mercury intoxication has brought about a number of significant changes in the glycogen content of liver, brain, kidney and muscle of *H. fossils*. It has been noted that 5 ppm of mercury increases the glycogen content of the liver, brain and muscle but the glycogen content decreases in liver and muscle as the concentration of mercuric salt increases to 25 and 50 ppm (Table I). Moreover, the kidney and brain responded differently with the mercury salt. The glycogen content of kidney decreased with the increase in the concentration of mercuric nitrate. However, in the brain, the glycogen contnet increased with 5 and 25 ppm of mercury intoxication and then decreases when the concentration was enhanced to 50 ppm

TABLE I

Changes in the tissue glycogen content of H. fossilis *by mercury intoxication*

Glycogen (µg/g of wet wt. of tissue)

	Control	Mercury intoxication in ppm		
		5 ppm	25 ppm	50 ppm
Liver	2827·50 ± 150·23	3262·50 ± 175·60	2283·75 ± 130·25	1450·00 ± 65·10
Muscle	1833·75 ± 75·25	2247·50 ± 140·38	1196·25 ± 116·39	833·75 ± 61·78
Brain	797·50 ± 56·36	1305·00 ± 85·67	1667·50 ± 132·36	652·50 ± 42·36
Kidney	543·75 ± 70·19	398·75 ± 40·37	181·25 ± 22·76	142·50 ± 30·36

Values are mean SE ± of 4 experiments.

The result of the present study shows that the mercury has different effects on glycogen content of various tissues of the fish. Lower concentrations of mercury elevated the glycogen content in liver, brain, and muscle tissues, whereas higher concentrations depleted the glycogen content in the kidney.

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---

*Request for reprints.

1. Goldwater, L. J., *Scientific American*, 1976, p. 3808.
2. Qayyum, M. A. and Ghayas Uddin, *All India Symposium on Advanced Ecology*, 1976, p. 68.
3. Llyod, R., *Ann. App. Biol.*, 1960, **48**, 84.
4. Skidmore, J. F., *J. Quart. Rev. Biol.*, 1964, **39**, 227.
5. —, *J. Exp. Biol.*, 1970, **52**, 481.
6. Slonim, A. R., *Prog. Fish Cult.*, 1973, **14**, 169.
7. Shaffi, S. A., and Habibullah, M., *Indian J. Exp. Biol.*, 1977, **15**, 309.

ON THE REGENERATION OF LABYRINTHINE ORGAN OF *ANABAS SCANDENS* (CUVIER)

DURING the course of an investigation on the function of the labyrinthine organ of *Anabas Scandens* (Cuvier), the author came across an interesting phenomenon of regeneration in the amputated regions of the labyrinthine organ. Since there was no previous record of regeneration of labyrinthine organ, the study was carried out in detail.

Anabas Scandens in the weight range from 10 to 25 gm were kept in the laboratory aquarium for over three months. They were fed regularly with boiled eggs. Three batches of fishes were isolated from the stock and maintained in individual jars. The labyrinthine organ was amputated in the first batch at the level of its origin. In the second batch, it was amputated leaving a small piece of labyrinthine organ tissue at its base. The third batch served as control.

For amputating, the fishes were anaesthetized using either ether or MS222 and the labyrinthine organ was removed by cutting off with sterilised scissors and scalpels in a sterilisation chamber. The amputated fishes were then allowed to recover and kept in water containing acroflavin (antibiotic).

Fishes amputated at the level of the origin from the first branchial arch (First batch) never regenerated. The wound healed without any regeneration blastema being formed. On the other hand, regeneration was observed in those fishes which have a little bit of labyrinthine organ tissue at their base (Second batch). From this it is inferred that the base of the labyrinthine organ acted as a regeneration territory[1] or regeneration field[1]. It took nearly three months for the regeneration of the 75% of the amputated parts. Preliminary histological studies revealed the presence of pillar-like epithelial cells, a characteristic feature of the labyrinthine organ of *Anabas Testudineus*[2]. Critical observations ruled out the possibility of heteromorphosis.

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1. Balinsky, B. I., *An Introduction to Embryology*, W. B Saunders Co., Philadelphia, U.S.A., 1970, p. 642.
2. Hughes, G. M. and Munshi, J. S. D., *Nature*, 1968, **219**, 1382.

MINIMUM LEVEL OF OXYGEN IN WATER FOR FISH SURVIVAL WITHOUT AIR BREATHING

THE oxygen content of natural waters varies between 0·04–10·0 ml/l and the solubility of oxygen in water is reduced by an increase in temperature[1]. Magid[2] has reported that a concentration 4·3 mg/l (3·10 ml/l) oxygen, *Clarias lazera* becomes restless, followed by an increase in the frequency of aerial breathing. In the present work an attempt has been made to study the minimum level of dissolved oxygen content in water for fish survival when their surfacing is prevented or thrir air breathing organ is not allowed to function.

Channa punctatus. Bloch and *Anabas testudineus* Bloch, both air breathing fishes, have been used here as material for the present study. The experiment was divided into two series. In the first series drowning experiment was conducted to see the effeciency of their gills for the adequate supply of oxygen to the body. It was found that if the water is well aerated (6·0–8·0 ml/l oxygen), *Channa punctatus* can survive indefinitely, but on the other hand it was observed that while larger specimens of *A. testudineus* (Weighing 25–62 g) were asphyxiated within an hour, others (weighing 6–18 g) did not die till the termination of the experiment after 10 days, which finding, tallies with that of Hora[3]. This is possibly indicative of the ability of *A. testudineus* weighing up to 18 g to survive indefinitely without contact with atmospheric air if the water is well aerated. In the next series of experiments, the aeration of the water of the container was stopped under the same experimental conditions to allow depletion of oxygen in water and to evaluate the exact level of oxygen for their survival. Both *A. testudineus* and *C. punctatus* died (100% mortality) within 2–3 h, as soon as the level of oxygen in water decreased to 2·93 and 2·79 ml/l respectively. The critical level of dissolved oxygen content was 3·26 and 3·10 ml/l respectively for *C. punctatus* and *A testudineus* at which 50% mortality took place in 35 minutes. At oxygen level of 3·52 ml/l there was no mortality at all, but below that level signs of slight restlessness were observed in both the species. This experiment was conducted at 30·5 ± 1° C.

It can, therefore, be inferred that if access to atmospheric air is not allowed, *C. punctatus* and *A. testudineus* cannot survive or get asphyxiated if the oxygen content of the water falls below 2·79 and 2·93 ml/l respectively. Brain annoxia may be the cause of immediate death.

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Gaya College, Gaya, A. K. Chanchal.
Bihar, India,
May 30, 1977.

1. Wood, W. D., *Principles of Animal Physiology*, Edward Arnold Ltd., London. 1968, 1.
2. Magid, A. M. and Abdel, *J. Zool. London*, 197 **163** (1), 63.
3. Hora, S. L., *Trans. Nat. Inst. Sci.*, 1935, 1, 1

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REACTIONS OF TRICYCLIC TETRADENTATE β-KETOIMINE COMPLEXES OF NICKEL(II) AND COPPER(II) IONS WITH ARYLDIAZONIUM CHLORIDES

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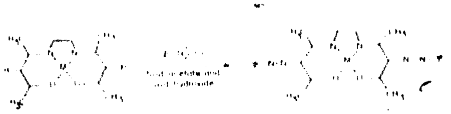
ELECTROPHILIC substitution reactions of coordinated β-diketones are well known[1-2]. Their reactivity stems mainly from the 'quasiaromaticity' of the chelate ring. Similar studies have also been extended to a few monovalent β-ketoimine complexes[3-8]. However, no comprehensive investigation of reactions of bivalent quadridentate Schiff base-metal complexes derived from β-diketones and 1,2-diamines has been done. Herein, we report a novel type of reaction of bivalent tricyclic tetradentate β-ketoimine complexes of Ni(II) and Cu(II) with phenyldiazonium chloride. The diazonium coupling reaction occurs on the γ-carbon atom of the chelated ring.

EXPERIMENTAL

N,N′-ethylene-bis(acetylacetoneimino)metal(II) complexes, M (AE) were prepared by the usual method[9].

A solution of M (AE) (0·01 mole) and sodium acetate (0·1-0·2 mole, to keep the pH around 2·6-3·0) in methanol was cooled to 0° C. This was treated with aqueous phenyldiazonium chloride (0·02 mole) dropwise with continuous stirring. The crystals of the complexes obtained were collected by filtration, washed with water and recrystallised from chloroform-methanol mixture. The reaction of M (AE) with phenyldiazonium chloride in equimolar ratio afforded monodiazo coupled complexes. The general pattern of the reaction is shown below.



Typical analytical data of some of the complexes is given in Table I.

TABLE I

Analytical data

Compound	C (%)	H (%)	N (%)	M (%)
Ni (AE) $(N_2ph)_2$	58·33	5·20	16·90	11·83
$C_{24}H_{22}N_6O_2Ni$	(58·9)	(5·35)	(17·18)	(11·90)
Ni (AE) $(N_2ph\text{-}p\text{-}CH_3)_2$	59·32	5·60	15·99	11·23
$C_{22}H_{33}N_6O_2Ni$	(60·30)	(5·80)	(16·20)	(11·34)
Ni (AE) N_2ph	56·21	5·63	14·39	15·19
$C_{18}H_{22}N_4O_2Ni$	(56·13)	(5·75)	(14·55)	(15·24)
Cu (AE) $(N_2ph)_2$	58·24	5·32	16·90	12·80
$C_{24}H_{26}N_6O_2$	(58·34)	(5·30)	(17·01)	(12·85)
Cu (AE) $(N_2ph\text{-}p\text{-}CH_3)_2$	59·20 (59·80)	5·31 (5·02)	16·36 (16·10)	12·29 (12·16)

Theoretical values are given in parentheses.

RESULTS AND DISCUSSION

The complexes gave satisfactory elemental analysis corresponding to the mono- and di-diazo coupled complexes. They are deeply coloured, crystalline, stable and have good solubility in both polar and non-polar solvents. Ni(II) complexes are diamagnetic implying planar geometry around the metal-ion. Cu(II) complexes are paramagnetic (*ca.* μ_{eff} 1·73 B.M.) which indicates square planar environment around Cu(II) ion.

The substitution of γ-CH carbon proton by the phenyl-diazonium group was confirmed by the absence of ir absorptions around 762 cm^{-1} (*s*) and 1190 cm^{-1} (*vw*) due to the out-of-plane and in-plane vibrations of the ring-CH respectively and also the absence of the ring -CH proton signal in the PMR spectra of the Ni(II) complexes. The ir spectra of both Cu(II) and Ni(II) complexes show ν (C=N) and ν (C—O) around 1645 cm^{-1} (*s*) and 1540 cm^{-1} (*s*) respectively. Slight increase in both of these frequencies are ascribed to the attachment of electronegative phenyldiazo group to the chelate ring.

The diazo-substituted β-ketoimine complexes of Ni(II) show no signal at 4·93 δ in the PMR spectra. This suggests that the substitution had occurred at the γ-carbon atom of the chelated ring. The signal due to the protons of the phenyl group occurs as a complex multiplet centered at 7·03 δ. The methyl protons resonate at 2·4 δ (-N-C)-CH_3 and 2·5 δ (-O-C)-CH_3. The signal due to the diamine skeleton protons gives a complex multiplet (possibly AA′ BB′ spin system) at 3·4 δ.

We now adduce evidence that the diazonium coupling reaction proceeds selectively. This is demonstrated by the synthesis of mono diazosubstituted β-ketoimine complexes. The ir spectra of these complexes show bands around 762 cm^{-1} (*s*) and 1190 cm^{-1} (*vw*) due to the out-of-plane and in-plane vibration of γ-(CH), respectively. Four bands are observed in the 1500-1700 cm^{-1} region of the ir spectra of thexe complexes. The bands at 1638 cm^{-1} (*s*), 1591 cm^{-1} (*s*) and 1566 cm^{-1} (*s*), 1520 cm^{51} (*s*) are respectively, due to ν (C=N) and ν (C-O) of the diazo-substituted and non-substituted chelated ring of the complexes. The Proton Magnetic Resonance Spectra of the Ni(II) complexes also show signals due to one γ-CH proton ($4 \cdot 9\delta$ singlet) and five protons of the phenyl group ($7 \cdot 33\delta$ multiplet), thereby confirming the nature of the substituted products. The generality of these reactions has been tested for both Ni(II) and Cu(II) complexes of the β-ketoimines.

It is worthwhile to point out that the diazonium coupling reaction presented here is not only novel but opens up a new area towards the synthesis of metal-ion containing dyes.

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1. Collman, J. P., *Adv. Chem. Ser.*, 1968, **37**, 78.
2. —, *Transition Metal Chemistry*, Marcel Dekker, New York, 1966, 2, 1.
3. Kluiber, R. W., *J. Am. Chem. Soc.*, 1960, **92**, 8889.
4. Collman, J. P. and Kittleman, E. T., *Inorg. Chem.*, 1962, **1**, 499.
5. Fujii, Y., *Bull. Chem. Soc., Japan*, 1970, **43**, 1722.
6. Kasahara, A. and Izumin, T., *Ibid.*, 1968, **41**, 2185.
7. Hipp, C. J. and Busch, D. H., *Inorg. Chem.*, 1972, **11**, 737.
8. — and —, *Ibid.*, 1973, **12**, 894.
9. McCarthy, P. J., Hovey, R. J., Ueno, K. and Martell, A. E., *J. Am. Chem. Soc.*, 1955, **77**, 5820.

ON THE MEASUREMENT OF RELATIVE OSCILLATOR STRENGTHS

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ABSTRACT

A method is indicated for determining the relative oscillator strengths for different lines using atomic absorption spectrophotometry. The method is applicable to a wide range of resonance lines which show similar hyperfine structure effects, and is free from systematic errors associated with the calibration of apparatus. The relative '*f*' values for the lines Ag-3383, Cu-3274, Mn-4030, Mn-3217, Sb-2127, Eu-3334, Tm-5307 and Yb-2673 have been estimated.

1. INTRODUCTION

COMMON methods for the measurement of oscillator strengths (*f*-values) include anomalous dispersion[1], absorption in optically thin layers[2] and emission measurements[3]. Ostroymenko and Rossikhin[4] used the method of line absorption for estimating Nlf taking into account differences in the emission/absorption width ratios (α-values). However, as stated by L'vov[5], this is unnecessary in the calculation of relative *f*-values. He proposed the measurement of these quantities from the analytical sensitivities obtained in flames. A slightly different approach for obtaining relative *f*-values for resonance absorption lines of an element showing similar hyperfine structure is indicated in this note.

2. PRINCIPLE

Consider the absorption of a beam of resonance radiation of frequency ν, through a cell containing a metal vapour at temperature T (and having vapour press p in atmospheres), such that similar situations exist in the emitting and absorbing gas and only heat motions of the atoms are taken into account. The peak absorption coefficient is defined as[6]:

$$K_{\nu}{}^{0} = \sqrt{\frac{\pi M}{2RT}} \cdot \frac{e^2}{m\nu_0} \cdot Nf \tag{1}$$

where the symbols have their usual meaning. On introducing the appropriate values of various constants and using ideal gas laws, one obtains

$$K_{\nu}{}^{0} = \frac{8 \cdot 512 \times 10^{15} pf \sqrt{M}}{\nu_0 T^{3/2}} \tag{2}$$

writing the absorbance in $I/I_0 = A_T$

$$A_T = K_{\nu}{}^{0} \cdot le^{-w^2} = K_{\nu}{}^{0} \cdot L \tag{3}$$

If a constant K_p is introduced such that,

$$K_p = \frac{\nu_0}{8 \cdot 512 \times 10^{15} f \sqrt{M L}} \tag{4}$$

Combination of (2), (3) and (4) gives,

$$p = K_p A_T T^{3/2} \tag{5}$$

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he measurements be repeated at a different freency ν, it is obvious that

$$K_p/K_p' = \frac{\nu_0}{\nu_0'}\cdot\frac{f'}{f} \tag{6}$$

ere the subscripts refer to each frequency.

he ratio K_p/K_p' is obtained in the following manner. he use of approximate Clausius Clapeyron's equation variation of vapour pressure with temperature es,

$$\log p = \alpha + \beta/T \tag{7}$$

ere alpha, beta are constants characterising the ment under study. Using (5) we obtain

$$\log K_p A_r T^{3/2} = \alpha + \beta/T \tag{8}$$

, log $A_r T^{3/2}$ should bear a linear relationship with . Such linear plots were obtained at various sonance lines of interest, by heating the absorption lls containing elements under investigation to various mperatures and determining corresponding absorances. The mean K_p/K_p' ratio could then be obtained om such plots and used to calculate the f-ratios for spective lines from relation (6).

3. Experimental

The element to be investigated, is sealed in an evacued quartz cell. This is heated in a suitable furnace to various desired temperatures, so as to obtain appreciable vapour pressure of the metal in the cell. The atomic absorbances by this vapour are measured at a series of temperatures using the respective resonance lines.

High purity elements (better than 99·9%) are used in the experiments. The temperatures are recorded with a thermocouple.

Linear plots of log $A_r T^{3/2}$ vs 1/T are obtained to evaluate mean K_p/K_p' for the respective resonance lines.

4. Results and Discussion

Figure 1 gives the plots obtained for the two resonance lines of silver, Ag-3281 and Ag-3383, and is typical of the plots obtained for estimating K_p/K_p' ratios in this work, for various resonance lines. The data obtained on these ratios and the calculated 'f' values for some resonance lines are given in Table 1

TABLE 1

Relative 'f' values obtained for some resonance lines

Element	Resonance line (Å)		K_p/K_p'	f'/f	f	
	Reference	Experimental			Present	Others
ver	3281	3383	2·33	2·27	0·21	0·215[8] 0·12[3] 0·22[9] 0·247[10]
pper	3247	3274	2·08	2·06	0·15	0·155[4] 0·16[11] 0·153[12]
anganese	2795	4030	13·95	9·65	0·060	0·056[17] 0·062[14] 0·33[3]
		3217	250·0	218·0	0·0026	0·0028[17] 0·00028[15]
ntimony	2068	2127	4·75	4·76	0·043	0·015[5] 0·042[18]
uropium	4594	3334	21·1	15·3	0·017	..
ıulium	3718	5307	13·4	19·1	0·011	...
tterbium	3988	2673	43·5	29·2	0·013	0·0167[20] 0·11[3]

Silver :

The relative 'f' values for silver Ag-3383 line calculates to 0·21 on the basis of 'f' for Ag-3281 as 0·45[7]. This compares with the value of 0·215 reported by Lawrence *et al.*[8] and the value 0·22 found by Hinnov and Kohn[9]. The value 0·12 given by Corliss and Bozmann[3] and 0·247 by Penkin and Slavenas[10] can be understood in the light of their 'f' values for Ag-3281.

Copper :

The 'f' value for Cu-3274 is in good agreement with the values given by Penkin[1], Ostroymenko and Rossikhin[4], L'vov[5] and by Bell *et al.*[11]. Noise[12] also reports a value which is close to the present value. Fermi[13] also found the ratio f_{3247}/f_{3274} to be 2·0 in good agreement with ours.

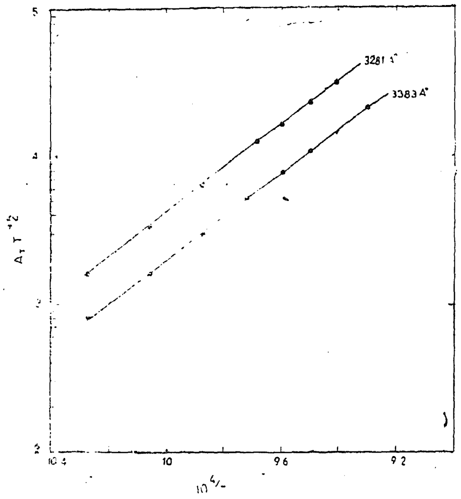

FIG. 1. Plots of log $A_T T^{3/2}$ *vs* 1/T for silver resonance lines. X-axis = 10^4/T, Y-axis = $A_T T^{3/2}$.

Manganese :

On the basis of 'f' value for Mn-2795 as 0·58, the same for Mn-4030 and Mn-3217 are 0·060 and 0·0026 respectively. The value for Mn-4030 agrees closely with those of Bell *et al*[11], and Huldt and Lagerquist[14], and is also in good agreement with other reported values[3, 9, 15–17]. The value reported by Hefferlin and Gearhart[15] for Mn-3217 is, however, lower by a magnitude.

Antimony :

The 'f' value for antimony Sb-2127, on the basis of that for Sb-2068 as 0·21 calculates to 0·043. This is three times higher as compared to that given by Corliss and Bozmann[3] but agrees closely with the value 0·042 reported by L'vov[18].

Europium :

If the 'f' value for Eu-4594 be assumed 0·26[7], which is taken from emission measurements, 'f' value for Eu-3334 is calculated as 0·017 from the ratio of respective K_p values. It is, however, significantly lower compared to that given by L'vov. However, the ratio 15·3 for f_{4594}/f_{3334} obtained by us is close to the value of 12·8 given by Komorovskii *et al.*[19]. The results are significantly at variance with those of Corliss and Bozmann[3].

Thulium :

The 'f' value obtained for Tm-5307 is 0·011. No data on this were available in the literature.

Ytterbium :

The value of 'f' for Yb-2673 is obtained as 0·013 and compares well with the results of Corliss and Meggars[20]. However the ratio of f_{3988}/f_{2673} differs very appreciably with that given by Corliss and Bozmann[3]. It appears that the value of 0·11 reported by these investigators for f_{2673} is due to a typographical error.

---

1. Penkin, N. P., *J. Quant. Radiat. Transfer*, 1964, **4**, 41.
2. King, R. B. and King, A. S., *Astrophys. J.*, 1935, **82**, 377.
3. Corliss, C. B. and Bozmann, W., *Experimental Transition Probabilities for Spectral Lines of Seventy Elements*, National Bureau of Standards, Washington DC (1962).
4. Ostroymenko, P. P. and Rossikhin, V. S., *Opt. Spec.*, 1965, **19**, 365.
5. L'vov, B. V., *Ibid.*, 1970, 28, 8.
6. Mitchell, A. C. G. and Zemansky, M. W., *Resonance Radiation and Excited Atoms*, Cambridge, The University Press, 1961.
7. L'vov, B. V., *Atomic Absorption Spectrochemical Analysis*, Adam Hilger, London, 1970.
8. Lawrence, G. M., Link, J. K. and King, R. B., *Astrophys. J.*, 1965, **141**, 293.
9. Hinnov, F. and Kohn, H., *J. Opt. Soc. Am.*, 1957, **47**, 156.
10. Penking, N. P. and I-Yu. Yu. Slavenas, *Opt. Spec.*, 1963, **15**, 3, 83.
11. Bell, G. D., Davis, M. H., King, R. B. and Routly, P. M., *Astrophys. J.*, 1959, **129**, 437.
12. Noise, N. L., *Ibid.*, 1966, **144**, 774.
13. Fermi, E.. *Z. f. Phys.*, 1930, **59**, 680.
14. Huldt, L. and Lagerquist, A., *J. Opt. Soc. Am.*, 1952, **42**, 142.
15. Hefferlin, R. and Gearhart, J., *J. Quant. Spect. Radiat. Transfer*, 1964, **4**, 9.
16. Morozova, N. G. and Startsev, G. P., *Opt. Spec.*, 1964, **17**, 174.
17. Ostrovskii. Yu. I. and Penkin, N. P., *Ibid.*, 1957, **3**, 193.
18. L'vov, B. V., *Opt. i. Spec.*, 1965, **19**, 282.
19. Komorovskii, V. A., Penkin, N. P. and Shabanova, L. N., 1968, **25**, 81.
20. Corliss, C. H. and Meggers, W. F., *J. Opt. Soc. Am.*, 1960, **50**, 1136.

KINETICS OF REACTIONS OF THIOUREA, PHENYLTHIOUREA, AND *p*-TOLYLTHIOUREA WITH SUBSTITUTED PHENACYL BROMIDES IN ETHANOL : STRUCTURE-REACTIVITY CORRELATION

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ABSTRACT

The kinetics of formation of thiazoles, from reactions of thiourea, phenylthiourea, and *p*-tolylthiourea with substituted phenacyl bromides, have been studied at four different temperatures (30–45°). The reactions are second order in the temperature range studied. Specific reaction rates and other thermodynamic parameters are reported. Isokinetic relationship is valid for the three reaction series and the values of isokinetic temperature (β) have been calculated. Justification of the Fairclough-Hinshelwood relationship implies that the reaction series is both energy and entropy controlled. The series is compatible with Exner's criterion showing that the same mechanism operates in each series. The rate constants at 40° have been correlated with the Hammett substituent constants, and the corresponding reaction constants computed. The values of the reaction constant are discussed in terms of the uncommon reactant in each series. Based upon the kinetic data and the discussion, two alternative mechanisms for the formation of thiazoles are proposed.

INTRODUCTION

THE reaction of an α-halo ketone with a thioamide has been the most important method for thiazole synthesis ever since it was originally introduced by Hantzsch and coworkers[1]. Ready access to a wide selection of the required reactants has made this method broadly applicable. By proper choice of reactants, thiazoles with alkyl, aryl, or heterocyclic substituents (attached to any of the three carbons of the thiazole nucleus) have been prepared. Thus when the thioamide reactant is a thiourea, a 2-aminothiazole results in excellent yields[1]. Some workers[2,3] have studied the thiazole synthesis kinetically. Okamiya[2] studied the reactions of thiobenzamides with phenacyl bromides. Behera *et al.*[3] reported the reactions of aryl thioureas with phenacyl bromide. The present investigation was undertaken to work out the mechanism of thiazole formation[4]. The effect of the change of substituents in either of the reactants has been studied.

EXPERIMENTAL

Thiourea (GR, Sarabhai M.) was used as such. Phenylthiourea and *p*-tolylthiourea were prepared and purified by the known methods[5]. Phenacyl bromides were prepared and purified by crystallization from ethanol[6-8]. Absolute ethanol was prepared by the usual method[9].

The rates of reaction were followed conductometrically in the temperature range 30–45° (± 0·05°). Equimolar solutions (M/50) of each of thiourea (or phenylthiourea, or *p*-tolylthiourea) and phenacyl bromide (or a substituted phenacyl bromide) were put into the conductivity cell and the conductivity measured at different time intervals. The temperature of the thermostat was finally raised to 60° to ensure completion of reaction. The infinity reading was taken after readjustment of the temperature. The reaction rates were calculated by the method described by Frost and Pearson[10]. The products of the reaction were in agreement with the standard u.v. spectrum of 2-amino-4-phenylthiazole.

RESULTS AND DISCUSSION

The reaction series obey second order rate equation, first order each in thiourea and phenacyl bromide. The second order rate constants at four different temperatures for the three reaction series are given in Tables I–III. The values are the average of at least two runs and are correct to ± 2%. The Arrhenius activation energy had an accuracy of ± 0·50 kcal/mole. The values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were calculated at 30°. Probable error limits for the $\Delta S^{\ddagger}$ values are ± 0·80 e.u.

The activation parameters are related by Fairclough–Hinshelwood relationship[11] and the isokinetic relationship[12]. The computed values of β and $\Delta H_0^{\ddagger}$ are recorded in the respective tables for the series.

The above two linear relationships imply that the reaction series are both energy and entropy controlled. In other words they are neither isoenthalpic nor isoentropic. Another conclusion that may be drawn is that all the derivatives in each series react with a similar mechanism.

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TABLE I

Second order rate constants and activation parameters for the reactions of thiourea and phenacyl bromides
[Thiourea] = M/100; [Phenacyl bromide] = M/100; Solvent: Absolute ethanol.

Phenacyl bromide	k litre mole^{-1} sec^{-1}				E_a kcal/mole	$\Delta H^\ddagger$ kcal/mole	$\Delta S^\ddagger$ e.u.	log PZ
	30’	35’	40’	45’				
p-Bromo	0·492	0·883	1·517	2·624	20·88	20·28	6·94	14·751
p-Chloro	0·374	0·684	1·222	2·118	21·91	21·31	9·79	15·375
Unsubstituted	0·225	0·486	0·897	1·607	23·42	22·82	14·02	16·298
p-Methyl	0·172	0·349	0·671	1·274	25·42	24·82	19·84	17·569
p-Methoxy	0·156	0·321	0·618	1·208	25·97	25·37	21·46	17·923

$\beta = 355\cdot4°$ K $\Delta H_0^\ddagger = 17\cdot82$ kcal/mole $\rho = 0\cdot718$

TABLE II

Second order rate constants and activation parameters for the reactions of phenylthiourea and phenacyl bromides
[Phenylthiourea] = M/100; [Phenacyl bromide] = M/100; Solvent: Absolute ethanol

Phenacyl bromide	k litre mole^{-1} sec^{-1}				E_a kcal/mole	$\Delta H^\ddagger$ kcal/mole	$-\Delta S^\ddagger$ e.u.	log PZ
	30’	35’	40’	45’				
p-Bromo	0·404	0·650	0·989	1·501	16·76	16·16	7·05	11·694
p-Chloro	0·327	0·529	0·821	1·282	17·27	16·67	5·79	11·970
Unsubstituted	0·245	0·406	0·641	1·014	18·13	17·53	3·52	12·465
p-Methyl	0·181	0·309	0·499	0·827	19·24	18·64	0·46	13·134
p-Methoxy	0·166	0·284	0·472	0·771	19·57	18·97	0·46	13·335

$\beta = 369\cdot6°$ K $\Delta H_0^\ddagger = 18\cdot8$ kcal/mole $\rho = 0\cdot592$

TABLE III

Second order rate constants and activation parameters for the reactions of p-*tolylthiourea and phenacyl bromides*
[*p*-Tolylthiourea] = M/100; [Phenacyl bromide] = M/100; Solvent: Absolute ethanol

Phenacyl bromide	k litre mole^{-1} sec^{-1}				E_a kcal/mole	$\Delta H^\ddagger$ kcal/mole	$-\Delta S^\ddagger$ e.u.	log PZ
	30’	35’	40’	45’				
p-Bromo	0·597	0·813	1·076	1·421	11·33	10·73	24·19	7·948
p-Chloro	0·462	0·646	0·881	1·211	12·28	11·68	21·57	8·521
Unsubstituted	0·327	0·473	0·679	0·944	13·42	12·82	18·49	9·193
p-Methyl	0·232	0·347	0·502	0·731	14·78	14·18	14·69	10·025
p-Methoxy	0·216	0·326	0·476	0·690	15·14	14·54	13·64	10·254

$\beta = 366\cdot9°$ K $\Delta H_0^\ddagger = 19\cdot6$ kcal/mole $\rho = 0\cdot658$

However, the Exner's criterion[13] was also applied to strengthen this conclusion.

The applicability of the Exner's criterion is difficult when the isokinetic temperature lies within the temperature range of kinetic measurements[4]. But as the β values in each of the present reaction series lie well away from the range of study, the above criterion is applicable. Plots of log k (40°) *versus* log k (30°) were drawn for all the three reaction series and straight lines were obtained. Thus the series are compatible with the Exner's criterion and follow identical mechanism (under the experimental conditions described).

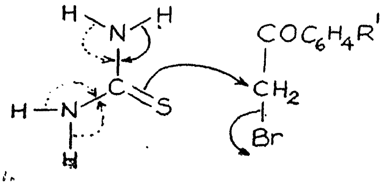
Structure–Reactivity Correlation

The satisfaction of the isokinetic relationship and the Exner's criterion enables us to correlate the structure with reactivity. The results of the structure-reactivity correlation with different scales of substituent constants were as follows :

Plots of log k against σ^+ values of Brown–Okamoto[14] were not linear, instead a curve with an upward concavity was obtained. The use of Yukawa–Tsuno equation[15] also was of little help, although the above concavity reduced gradually as the weightage to the conjugative effects was decreased. The best possible fits were achieved only with the Hammett σ values[16]. This shows that the effects of the substituents in these reaction series are of the same nature as their acid-strengthening effect on benzoic acids, and that there are no additional resonance effects. Plots of log k(40°) *versus* σ were drawn. The slopes (calculated by the method of least squares) of the lines give the reaction constant (ρ) values for the three reaction series.

The ρ values (given in the respective Tables) are positive, showing that the reactions proceed with a mechanism in which electrons are withdrawn by the substituents from the reaction site (methylene of phenacyl bromide). Thus, the reaction site is made more and more electrophilic as the substituents are changed in the order p-CH_3O, p-CH_3, H, p-Cl, p-Br. The values of ρ being less than unity, the effects of substituents in these reactions are not as strong as their corresponding acid-strengthening effect on benzoic acids.

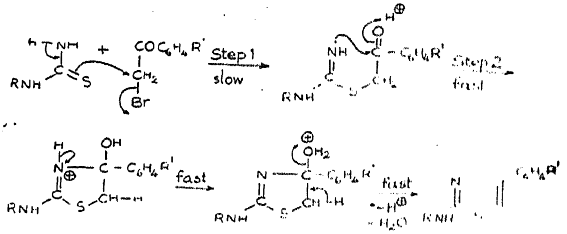
The variation of ρ values among the three reaction series reflects the response (to accept attack) of the reactant other than phenacyl bromide (namely, the thioureas). Among the three thioureas, the ρ value for the reaction of unsubstituted thiourea with phenacyl bromides is the highest. This shows that the reaction proceeds more conveniently with thiourea than with the substituted thioureas. There are two possible explanations. Firstly, there are four H atoms in thiourea and any one of them can leave as a proton and aid in the initial attack of sulphur on the methylene of phenacyl bromide. Thus



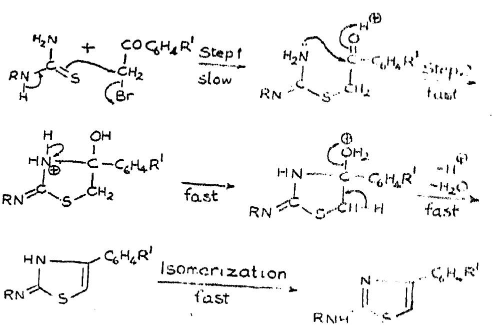
Only one H leaves (shown by solid arrow). The dashed arrows indicate the other three possibilities. In the case of the substituted thioureas, the position of one of the H atoms is occupied by the substituent and the probability of assistance by H is reduced to 75%. Secondly, it is possible that the smaller size (or lower molecular weight) of thiourea makes it more responsive to attack. It can easily orient itself according to the mechanistic requirements of the reaction. In view of both these possibilities the higher ρ value of p-tolylthiourea reaction as compared to that of the reaction of phenylthiourea, can be understood by taking into account the inductive effect. The inductive effect of p-tolyl is more positive than that of phenyl group. This makes the sulphur more nucleophilic and the initial attack is favourably accepted.

Mechanism

In the light of the foregoing discussions, the mechanisms shown in Scheme 1 and Scheme 2



SCHEME 1



SCHEME 2

are proposed. These alternative mechanisms are kinetically indistinguishable. The rate being second order, the first step is the slow and hence the rate-determining step. The probable subsequent steps are unimolecular and are fast, so that they do not affect the kinetics.

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1. Hantzsch, A. *et al.*, *Liebigs Ann. Chem.*, 1888, 249, 1, 31; 1889, 250, 257; 281; 1890, 259, 228, 253.
2. Okamiya, J., *Nippon Kagaku Zasshi*, 1965, 86, 315.
3. Behera, G. B., Acharya, R. C. and Rout, M. K., *Indian J. Chem.*, 1973, 11, 81.
4. Bhatti, A. M., *Ph.D. Thesis*, Punjabi University, Patiala, 1975.
5. Kurzer, F., *Organic Syntheses*, Vol. XXXI, Edited by R. S. Schreiber, John Wiley, London, 1951, p. 21.
6. Cowper, R. M. and Davidson, L. H., *Ibid.*, Coll. Vol. II, Edited by A. H. Blatt, John Wiley, New York, 1961, p. 480.
7. *Beilsteins Handbuch der Organischen Chemie*, Band VII, Springer-Verlag, Berlin, 1925, pp. 285, 309.
8. *Ibid.*, Band VIII, 1925, p. 89.
9. Vogel, A. I., *A Textbook of Practical Organic Chemistry*, Longmans, London, 1961, p. 161.
10. Frost, A. A. and Pearson, R. G., *Kinetics and Mechanism*, Wiley Eastern, New Delhi, 1970, p. 36.
11. Fairclough, R. A. and Hinshelwood, C. N., *J. Chem. Soc.*, 1937, p. 538.
12. Leffler, J. E., *J. Org. Chem.*, 1955, 20, 1202.
13. Exner, O., *Coll. Czech. Chem. Commun.*, 1964, 29, 1094.
14. Brown, H. C. and Okamoto, Y., *J. Am. Chem. Soc.*, 1957, 79, 1913; 1958, 80, 4979.
15. Yukawa, Y. and Tsuno, Y., *Bull. Chem. Soc. Japan*, 1959, 32, 971.
16. McDaniel, D. H. and Brown, H. C., *J. Org. Chem.*, 1958, 23, 420.

STUDIES IN RUBIACEAE—III

Structure and Development of Ovule and Seed of *Oldenlandia gracilis* DC.

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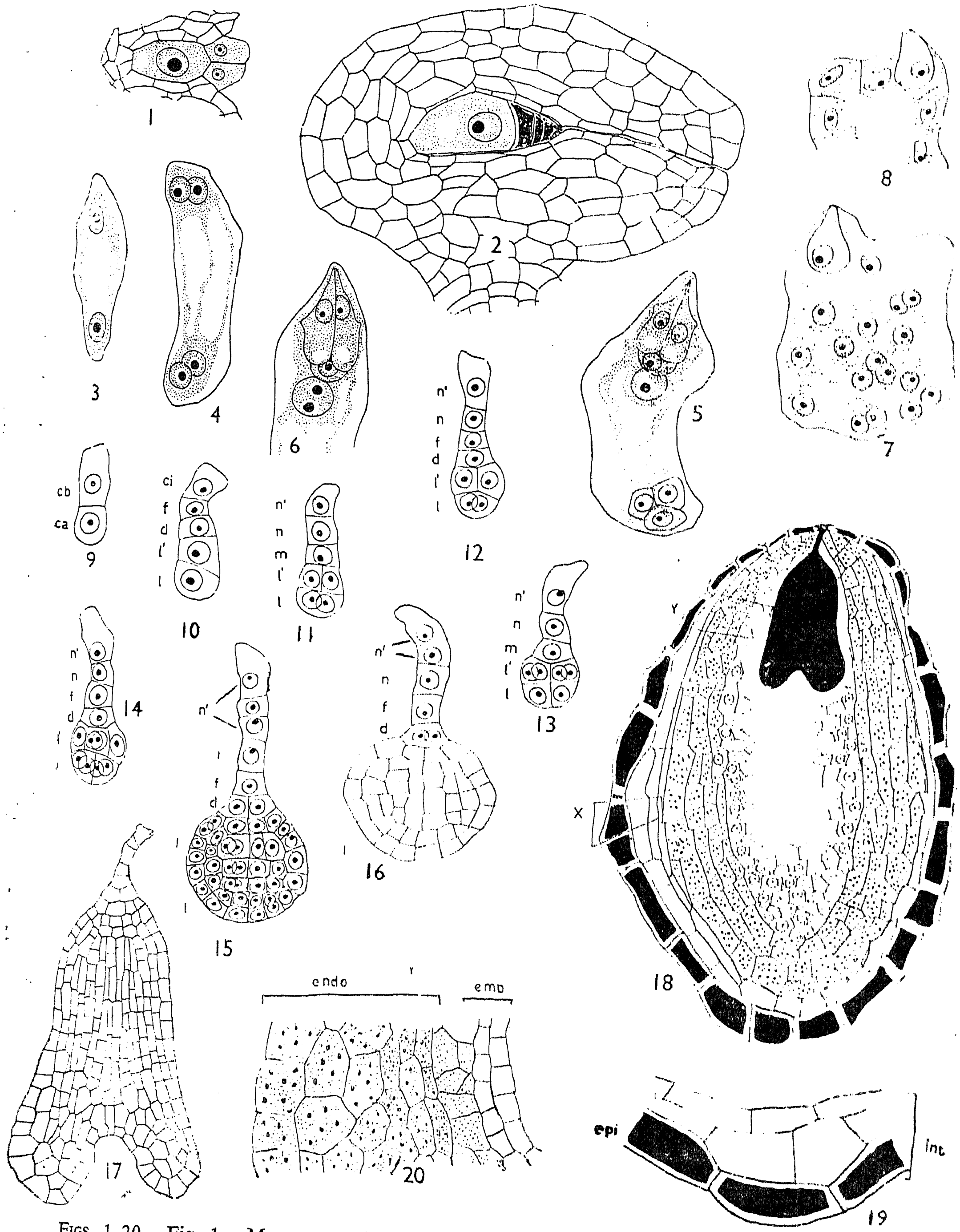
A REVIEW[6] of the early embryological literature on Rubiaceae reveals that *Oldenlandia*, a genus with several species, have received little attention except for the contributions of Raghavan and Rangaswami[5] on *O. alata* and Farooq[2-3] on *O. corymbosa* and *O. nudicaulis*. There is thus scope for further work on more species of this genus. The present investigation deals with the structure of the ovule, development of the female gametophyte, endosperm, embryogeny and structure of seed of *O. gracilis* DC.

O. gracilis are slender erect herbs with small white flowers and elongate capsules. Flower buds and fruits were collected in July from the National Park, Bannerghatta, Bangalore District and fixed in Formalin-acetic-alcohol; *n*-Butanol series was used for dehydration and infiltration; Paraffin sections were cut at 8–10 µm and stained with Heidenhain's haematoxylin and counterstained with erythrosin.

The ovary is inferior, bicarpellary syncarpous and bilocular with numerous hemianatropous, unitegmic and tenuinucellate ovules borne on axile placentae. The nucellus is highly reduced and appears as a small papillate growth on the placenta. The integument initiates its growth as the female archesporium, represented by a single cell, differentiates hypodermally in the nucellus. The archesporial cell functions directly as the megasporocyte which lies directly under the nucellar epidermis consisting of only two cells (Fig. 1). The nucleus of the megasporocyte undergoes meiotic divisions resulting in a linear tetrad of megaspores (Fig. 2). By this time, the integument grows around crushing the nucellar epidermis. A narrow micropylar canal is formed. The integument which is 5–7 cells thick does not form an integumentary tapetum around the embryo sac.

The megaspore at the chalazal end of the tetrad functions while the upper three degenerate (Fig. 2). The functional megaspore after undergoing three successive free nuclear divisions gives rise to the 8-nucleate embryo sac (Figs. 3–5). In the mature embryo sac, the synergids are hooked. The two polar nuclei fuse before fertilization to form a large secondary nucleus which lies abutting the egg cell. The antipodals are organised into large cells and degenerate soon after fertilization. The embryo sac at the time of fertilization is slightly curved (Fig. 6).

Fertilization is porogamous. One of the synergids is destroyed during the entry of the pollen tube into the embryo sac, The primary



FIGS. 1–20. Fig. 1. Megasporocyte and the nucellus. Fig. 2. Ovule showing megaspore tetrad. Figs. 3–6. Stages in the development of embryo sac. Figs. 7 and 8. Stages in the development of endosperm. Figs. 9–17. Stages in the development of embryo. All figures, × 1,200 (except Figs. 18, 19 and 20). Fig. 18. L.S. of seed showing testa, endosperm and embryo, × 480. Fig. 19. Part of seed showing testa, × 1,600. Fig. 20. Part of seed showing endosperm, × 1,600. (*ca, cb,* products of zygote ; *ci, m,* products of *cb* ; *d, f,* products of *m* ; *n, n′,* products of *ci* ; *l* and *l′*, derivatives of *ca* ; *end, endosperm* ; *emb*, embryo.)

endosperm nucleus divides earlier than the zygote. No wall is laid down between the daughter nuclei. The two nuclei thus produced move apart and enter a series of simultaneous free nuclear divisions to produce about sixteen nuclei in the much vacuolated cytoplasm (Fig. 7). Shortly after the nuclear divisions stop, the cytoplasm with the enclosed nuclei moves centrifugally forming a peripheral layer enclosing a central vacuole. Simultaneous wall formation commences first towards the periphery (Fig. 8) and soon the entire fertilized embryo sac becomes cellular (Fig. 18).

Division of the zygote takes place as cell walls are laid between the free endosperm nuclei. Its segments transversely resulting in a terminal cell *ca* and a basal cell *cb* (Fig. 9). The basal cell divides transversely to form two superposed cells, *m* and *ci*. Each of these cells divides transversely to produce four cells, *d*, *f*, *n* and *n'* basipetally. Meanwhile, the terminal cell *ca* also divides transversely, forming cells *l* and *l'* (Fig. 10). Longitudinal divisions in these cells give rise to the quadrants, octants and later stages (Figs. 11–14). Further divisions in the tier *l'* take place more rapidly than in the tier *l* (Fig. 14). Eventually, the embryo becomes globular, heart-shaped and ultimately, dicotyledonous (Figs. 15–17). The various regions of the differentiated embryo are the following: Cotyledons and stem apex from *l*; hypocotyledonary region and all parts of the root except the root cap from *l'*; root cap and part of the suspensor next to the embryo from *m*; remaining portion of the suspensor from *ci*. The basal suspensor cell tends to be vesicular with dense cytoplasm and large nucleus. The suspensor remains persistent for a long time. The embryogeny of *Oldenlandia gracilis* conforms to the Nicotiana variation of the Solanad type[4].

The cells of the endosperm contain plenty of starch grains (Figs. 18 and 20). The developing embryo consumes part of the endosperm around its vicinity forming a central cavity. In the mature seed, the endosperm persists in the form of five or six layers. The inner layers of the integument are crushed by the developing endosperm. The testa is represented by three layers, the outermost of which shows tangentially-stretched, tannin-filled cells (Fig. 19).

Fagerlind[1] has shown probable lines of reduction in the archesporium and nucellus in Rubiaceae. The nucellus and the archesporium in *Oldenlandia gracilis* conform to his[1] *Oldenlandia* type. An integumentary tapetum is not differentiated in this species. Hooked synergids noticed here are also reported in *O. nudicaulis*. Non-nucleate cytoplasmic vesicles formed during endosperm development in *O. corymbosa* and *O. nudicaulis* were not observed in this species. The suspensor of the embryo is five-celled in this while it is less than five-celled in *O. corymbosa* and *O. nudicaulis*. The basal suspensor cell appears to be feebly haustorial. A similar basal suspensor cell has been noticed in *Richardsonia, Leptodermis* (Fagerlind[1]), and *Oldenlandia nudicaulis* (Farooq[3]).

ACKNOWLEDGEMENT

Our grateful thanks are due to the Principal, St. Joseph's College, Bangalore, for his encouragement.

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1. Fagerlind, F., *Acta Hort. berg.*, 1937, **11**, 194.
2. Farooq, M., *Curr. Sci.*, 1958, **22**, 280.
3. — and Inamuddin, M., *J. Indian bot. Soc.*, 1969, **68**, 166.
4. Johansen, D. A., *Plant Embryology*, 1950.
5. Raghavan, T. S. and Rangaswami, K., *J. Indian bot. Soc.*, 1941, **20**, 341.
6. Shivaramiah, G. and Sundara Rajan, S., *Proc. Indian Acad. Sci.*, 1973, **77 B**, 19.

EFFECT OF LIPID PEROXIDATION ON ACID PHOSPHATASE OF RAT TISSUES

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THE toxic effect of peroxides produced by peroxidising unsaturated fatty acids on certain cellular functions has been well documented[1-3]. Bernheim *et al.*[4] have shown that fatty acid peroxides inhibit some oxidative enzymes; Wills[5] has reported that different enzymes like urease, glyoxalase and papain are inactivated by preincubation with linoleic acid emulsion. Acid phosphatase is a typical lysosomal enzyme and as such is widely used as a marker enzyme for following lysosomal damage[6]. The amount of enzyme leaked out into the medium gives the damage of the lysosomal membranes. It has been established that lipid peroxidation of the organelles' membranes is the basic mechanism for the increased leakage of their constituent enzymes like acid phosphatase, cathepsin, etc., into the medium[6-8]. However, whether lipid peroxidation *per se*, has any direct effect on acid phosphatase of the tissues has not been studied so far, and, in the present study we report the direct effect of peroxidising linoleic acid on the activity of acid phosphatase of rat tissue homogenates, – brain, liver, kidney, heart, spleen and serum. Lipid peroxidation is monitored by estimating the malonic dialdehyde formed during the peroxidation by thiobarbituric acid[9].

Albino rats of both sexes (weight range 100–130 gm) were starved overnight and sacrificed by heart puncture under light ether anaesthesia. Blood and the various tissues were immediately removed and the serum separated in the cold. The tissues were washed with ice cold saline and the homogenates prepared in cold 0·25 M sucrose in a glass Potter–Elvehjem–type homogeniser and filtered through a cheese cloth. The cell debris were removed by slow speed centrifugation in the cold and the supernatant used as enzyme sources. No attempt was made to isolate the lysosomal or mitochondrial pellets, since our interest was only to study the changes in the total enzyme activity of the tissues. Pure linoleic acid (10 gm) in 100 ml of petroleum ether (60–80°) was first passed through an alumina column and the eluant was freed of contaminated peroxides by repeated extractions with methanol : water (3 : 1) and the petrol layer containing linoleic acid was concentrated under nitrogen at 40° C. The oily residue was treated with 0·1 N NaOH to pH 7·0 and made up to 10% emulsion with distilled water and stored in freezer. Linoleic acid hydroperoxide (LAHPO) was prepared following the method of O'Brein[10] and finally 10% (v/v) emulsion was prepared using 1% Tween–80 in water and stored in the freezer. Linoleic acid and its hydroperoxide emulsion were prepared fresh every week. The linoleic acid thus purified gave negligible pink colour when treated with TBA indicating that the preparation was essentially free of peroxides. Acid phosphatase was assayed[11] using sodium phenyl phosphate as substrate. The assay system essentially contained 300 μ moles of acetate buffer (pH 5·0), 30 μ moles of the substrate and suitable amount of the enzyme in a total volume of 6 ml. The incubation was at 37° C for 60 min. and the phenol liberated estimated by Folin–Ciocaultaue reagent. Suitable controls including one for spontaneous hydrolysis were run for every experiment. To study the effect of linoleic acid emulsion or LAHPO emulsion, 1 ml of the required emulsion was included in the assay system and aliquots of 1 ml each were withdrawn at the beginning and end of the incubation for TBA measurement as dsecribed by Krishnamurthy and Bieri[9]. When LAHPO was present in the assay system, the initial TBA index was high (about 0·6) but no significant increase was found at the end of 60 min. incubation. Results are summarised in Table I. It may be noted that dilute tissue homogenates catalyse the peroxidation of linoleic acid (sodium salt) emulsion at pH 5·0 confirming the earlier report of Wills[12]. Even brain homogenates, which can peroxidise by itself, increases the rate of peroxidation of linoleic acid while serum inhibits the same as reported by Barber[13]. The acid phosphatase activity of the tissues is resistant to peroxidising lipid, in the case of liver, kidney and spleen while both brain and heart enzymes are inhibited up to about 40–50% when peroxidising linoleic acid is included in the reaction mixture when α-tocopjerol or a

TABLE I

Linoleic acid peroxidation and its hydroperoxide and acid phosphatase of rat tissues

Tissues	Activity	Peroxidation (TBA index increase)	% inhibition of activity
Brain (10)	63 ± 10·1	0·07 ± 0·01	..
Brain + LA	33 ± 5·2	0·28 ± 0·06	48
Brain + LAHPO	30 ± 6·1	..	52
Heart (8)	72 ± 8·2	0·03	..
Heart + LA	41 ± 7·2	0·26 ± 0·04	43
Heart + LAHPO	38 ± 5·3	..	47
Liver (6)	170 ± 13·2	0·06	..
Liver + LA	173 ± 14·8	0·25 ± 0·03	Nil
Liver + LAHPO	168 ± 12·8	..	Nil
Kidney (6)	220 ± 16·2	0·04	..
Kidney + LA	216 ± 15·1	0·16 ± 0·04	Nil
Kidney + LAHPO	222 ± 17·2	..	Nil
Spleen (5)	260 ± 14·2	0·05	..
Spleen + LA	250 ± 12·1	0·42 ± 0·06	Nil
Spleen + LAHPO	246 ± 16·1	..	Nil
Serum (3)	46 ± 4	Nil	..
Serum + LA	31 ± 6·1	Nil	33
Serum + LAHPO	30 ± 3·5	..	32

Values are mean ± S.E.
Number in parenthesis indicates observations.
Activity — μ moles of phenol liberated/hour/g tissues.
LA — Linoleic acid (Na salt).
LAHPO — Linoleic acid hydroperoxide.

synthetic antioxidant, DPPD (Diphenyl-P-Phenylene diamine) incorporated in the incubation medium, both lipid peroxidation (TBA pigment formation) and inhibition of the enzyme activity are decreased, although 100% recovery of the original activities were not obtained (To be published elsewhere). Serum acid phosphatase however showed a different picture, in as much as, although serum itself inhibited peroxidation of linoleic acid, the enzyme activity was inhibited by about 30% in presence of linoleic acid. It may be concluded therefore that the acid phosphatase activity of the various tissues may be differentiated according to: (*a*) liability against peroxidation damage *per se* (brain and heart enzymes), (*b*) not effected by such peroxidation (liver, kidney and spleen enzymes) and (*c*) non-specific inhibition by linoleic acid without peroxidation (serum enzyme). These results are in agreement with the more generalised observations of Wills[5], who has shown 4 groups of enzymes: (1) no effect by both saturated and unsaturated acids, (2) inhibited by both saturated and unsaturated acids, (3) inhibited only by unsturated acid and (4) inhibited more by unsaturated acid than saturated acid. Our results show that acid phosphatase, one of the marker enzymes studied to, determine lysosomal damage, which is implicated as due to free-radical effect by peroxidising lipid[1], is itself inhibited by such peroxidation, in brain and heart. The different degrees of stability towards peroxidation damage of brain and heart enzymes as against those of liver, kidney and spleen do not, however correlate with the rates of the tissue catalysis of linoleic acid peroxidation since spleen gives the maximum peroxidation in agreement with the report of Wills[12], although its enzyme activity is not affected. Similarly both liver and kidney homogenates actively catalyse peroxidation, while enzyme activity remains unaffected. Hence the enzyme stability of various tissues has also to be taken into consideration when studies on lysosomal acid phosphates are conducted. The effect of preformed linoleic acid hydroperoxide closely follows the effect of actively forming peroxides for linoleic acid in its effect on the activity of phosphatases of the various tissues.

1. Tappel, A. L., *Fed. Proc.*, 1973, 32, 1874.
2. Victoria, E. J. and Barber, A. A., *Lipids*, 1969, 4, 582.
3. McKnight, R. C., Hunter, F. E. and Oehlert, W. H., *J. Biol. Chem.*, 1965, 240, 3439.
4. Bernheim, F., Wilbur, K. M. and Kenaston, C. B., *Arch. Biochem.*, 1952, 38, 177.
5. Wills, E. D., *Biochem. Pharmacol*, 1961, 7, 7.
6. Baccino, F. M., Rita., G. A. and Dianjani. M. G., *Enzymologia*, 1965, 29, 169.
7. Chio, K. S. and Tappel, A. L., *Biochemistry*, 1969, 8, 2827.
8. Tappel, A. L., Swant, P. L. and Shibko, S., In: *Lysosomes*, Ed., by A. V. de Reuck and M. P. Cameron, London and Churchill, 1963, p. 78.
9. Krishnamurthy, S. and Bieri, J. G., *J. Nutrition*, 1962, 77, 245.
10. O'Brien, P. J., *Can. J. Biochem.*, 1969, 47, 485.
11. King, E. J., *Microanalysis in Medical Biochemistry*, London and Churchill, 1951, p. 69.
12. Wills, E. D., *Biochem. J.*, 1966, 99, 667.
13. Barber, A. A., *Arch. Biochem. Biophys.*, 1961, 92, 38.

OBITUARY

S. R. S. Sastry

We regret to announce the sad demise of Mr. S. R. S. Sastry on 23rd September 1977 due to pulmonary embolus. Mr. Sastry joined Current Science in 1938 and rendered it loyal and devoted service for nearly 40 years. He became the Manager in 1949 and Publisher in 1962. He contributed much to the growth and success of the journal.

He is mourned by his wife, his six children, and a large number of friends and scientists associated with Current Science.

LETTERS TO THE EDITOR

PRELIMINARY CRYSTALLOGRAPHIC DATA OF MANGANOUS URANYL ACETATE HEXAHYDRATE

YELLOW single crystals of manganous uranyl acetate hexahydrate were grown by the evaporation of an aqueous solution containing equimolar proportions of hydrated manganous and uranyl acetates. The preliminary cell parameters, obtained from oscillation, rotation and Weissenberg pictures, were refined by the method of least-squares using several high angle reflections in the (h0l) and (hk0) Weissenberg photographs. The space group was obtained from the systematic absences. The density was measured by the method of floatation using a liquid mixture of bromoform and carbon tetrachloride. The crystal data are presented in Table I.

TABLE I

Crystal Data of Manganous uranyl acetate hexahydrate

Manganous uranyl acetate hexahydrate $MnUO_2(CH_3COO)_4 \cdot 6H_2O$
System: orthorhombic
$a = 20 \cdot 133$ (16), $b = 12 \cdot 602$ (11), $c = 7 \cdot 790$ (6) Å
Volume of the unit cell $= V = 1976 \cdot 3$ Å^3
Formula Weight = F. W. = 667·27 gm.
No. of molecules in the unit cell $= Z = 4$
Density (observed) $= 2 \cdot 23$ gm. cm^{-3}
Density (Calculated) $= 2 \cdot 24$ gm. cm^{-3}
Linear absorption coefficient μ (CuKα) $= 344 \cdot 9$ cm^{-1}
Space group : $Pna2_1$ or $Pnam$ (Conditions: 0kl; $k + l$ = even h0l; h = even 00l; l = even)

Three dimensional X-ray intensity data (hkL, L = 0 to 6; hKl, K = 0 and 1) were collected by the multiple film equi-inclination Weissenberg procedure using CuKα radiation. Spot shape, Lorentz-polarization and absorption corrections were applied in the usual way. Using a three dimensional Patterson synthesis computed with 1197 observed reflections, the heavy atom, uranium, was located at (0·17, 0·043, 0·25). The complete structure will be elucidated by Fourier methods and reported later.

We are grateful to Professor K. S. Chandrasekaran for his kind encouragement and one of us (RKR) is thankful to the University Grants Commission for the award of a Junior Research Fellowship.

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Madurai 625 021, *July* 18, 1977.

ISOLATION OF MASLINIC ACID FROM *CALLICARPA ARBOREA* ROXB.

Callicarpa arborea Roxb. (N.O. Verbenaceae) is a moderate sized tree with ever green leaves[1], locally reputed to be a cure for cutaneous diseases[2]. Extraction of the heartwood with hexane and methanol yielded only small quantities of extractable material (hexane extract: 0·2%, methanol extract: 0·4%). On careful column chromatography over silica gel, hexane extract gave rise to β-sitosterol only and methanol extract furnished oleanolic acid. Both were identified by direct comparison with authentic specimens and also through their derivatives.

The leaf powder was similarly extracted with hexane, chloroform and ethyl acetate. Careful chromatographic separation on alumina using hexane and hexane-benzene mixtures, yielded lupeol[3], m.p. 210–212°; $(\alpha)_D + 23°$; acetate, m.p. 212–215°; $(\alpha)_D + 45°$; β-amyrin[4], m.p. 199°; $(\alpha)_D + 88°$; acetate, m.p. 235°; $(\alpha)_D + 86°$ and β-sitosterol, m.p. 134–135°; $(\alpha)_D - 35°$; acetate, m.p. 125–127°; $(\alpha)_D - 37°$. All of them were identified by direct comparison with authentic samples and through their derivatives like acetates and benzoates.

The chloroform and ethyl acetate extracts were chromatographed on silica gel separately, using benzene-ethyl acetate mixture (1: 1) as the eluent. In both the extracts oleanolic acid[5], m.p. 304–306°, $(\alpha)_D + 76°$; methyl ester acetate, m.p. 216–218°; $(\alpha)_D + 56°$, was found and identified through its methyl ester acetate. In the ethyl acetate extract, besides ursolic acid[6], m.p. 275–278°; $(\alpha)_D + 63°$; methyl ester acetate, m.p. 168–169°; $(\alpha)_D + 58°$, identified through its methyl ester acetate, there was a more polar triterpene which analysed for a dihydroxy triterpene mono carboxylic acid.

It crystallised as small needles from methanol, $C_{30}H_{48}O_4$, m.p. 266–269°, $(\alpha)_D + 40°$ diacetate, m.p. 234–235 ; $(\alpha)_D + 32°$; a methyl ester diacetate, m.p. 180–182°, $(\alpha)_D + 35°$; I.R. ν_{max}^{nujol} 1746, 1720 cm^{-1}. From the I.R. spectrum of the later derivative, it appears that the compound contains two easily acylable hydroxyls. The parent triterpene carboxylic acid reacted with one mole of periodate in 9·5 hours, suggesting that it could contain 1, 2-*trans* glycol system[7]. The methyl ester diacetate furnished a M^+ ion at m/e 570 and Retro Diels Alder fragments at m/e 262 (100%) and m/e 308 (3·8%). These two fragments confirm that ring system *A* carries the glycol. The NMR spectrum of the methyl ester diacetate also confirms the *trans* glycol system. δ 2·0 (s)-3H, 2·08 (s)-3H for two $-COCH_3$; δ 3·68 (s)-3H for $-COOCH_3$; δ 4·85 (d)-1H (J = 10 Hz) for C_3-αH; δ 5·15 (m)-1H for C_2-βH; δ 5·31 (t)-1H for C_{12}-H. All these spectral measurements suggest that the dihydroxy triterpene mono carboxylic acid could be maslinic acid, 2α, 3β-dihydroxy olean-12-en, 28-oic acid. The identification was finally confirmed by comparison with an authentic sample, earlier isolated in these laboratories from *Eugenia jambolona*[6]. It was first reported in *Crataegus oxyacantha*[8] and also from several plants of Myrtaceae[9,10].

One of the authors (VLN) is thankful to C.S.I.R. for a Junior Research Fellowship.

Department of Chemistry, A. S. R. Anjaneyulu.
Andhra University, V. Lakshminarayana.
Waltair, *July* 22, 1977. L. Ramachandra Row.

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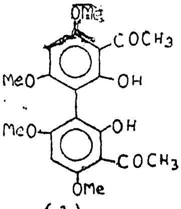
1. Gamble, J. S. and Fischer, C. E. C., *Flora of Madras*, 1967, **2**, 764.
2. *Glossary of Indian Medicinal Plants*, C.S.I.R., 1956, P. 45.
3. Sundara Ramayya, T. and Ramachandra Row, L., *Ind. J. Appl. Chem.*, 1963, **26**, 29.
4. Row, L. R., Sastry, C. S. P. and Murthy, P. S. N., *Curr. Sci.*, 1966, **35**, 146.
5. Subba Rao, G. S. R. and Row, L. R., *Tetrahedron*, 1962, **18**, 827.
6. Ph.D. thesis of Gopala Rao, M., submitted to Andhra University 1968,
7. Subba Rao, G. S. R. and Row, L. R., *Tetrahedron, Letters*. 1960, **27**, 12–16.
8. Tschesche, R. and Fugmann, R., *Chem. Ber.*, 1951, **84**, 810.
9. Arthur, H. R. and Hui, W. H., *J. Chem. Soc.*, 1954, p. 1403.
10. Potts, K. T. and Roy, S. K., *Aust. J. Chem.*, 1965, **18**, 767.

A NEW SYNTHETIC BIFLAVONE

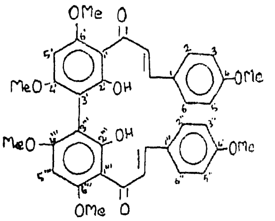
Biflavones are usually synthesised by the simple or crossed Ullmann condensation of the appropriate iodoflavones[1,2], the dehydrogenation of the bichalcones[3], double Baker-Venkataraman transformation of the suitable biphenyl derivatives[4,5] followed by ring closure and the Allan-Robinson condensation of the biphenyl derivatives[6].

The Algar-Flynn-Oyamada (AFO)[7,8] oxidation of chalcones gives flavonols through the intermediacy of flavanonols. In the present communication, we wish to report the synthesis of the biflavone (3) by the oxidation of the bichalcone (2).

Reaction of 2, 2′-dihydroxy-4, 4′,6,6′-tetramethoxy-3,3′-diacetylbiphenyl[4,5] (1) with anisaldehyde in presence of alkali produced the bichalcone[5] (2). Reaction of alkaline hydrogen peroxide on the bichalcone (2) in pyridine at 65° gave I–3, II-3-dihydroxy-I-4′, II-4′, I-5, II-5, I-7, II-7-hexamethoxy [I-8, II-8] biflavone (3); m.p. 228–229°. IR-spectrum of the biflavone (3) showed absorption at 1630 (chelated carbonyl) and 3300 cm^{-1} (OH). Mass spectrum showed molecular ion peak at m/e 654 and the general fragmentation pattern was typical of a C-C-linked biflavone. (3) on acetylation with acetic anhydride and pyridine gave the diacetate (4), m.p. 175–176°. IR-spectrum of compound (4) showed absorption at 1640 (C=0) and 1780 cm^{-1} (acetate). Acetylation of the 3-hydroxyl produced the shift (+ 10 cm^{-1}) to higher wavelength. NMR spectrum of the diacetate (60 MHz, $CDCl_3$, TMS as internal standard, δ ppm); 1·95 (s, 2 OAc), 3·7 (s, 2 OMe), 3·80 (s, 2 OMe), 4·1 (s, 2 OMe), 6·5 (s, 2 H), 6·6 (d, J = 10 Hz, 4 H) and 7·2 (d, J = 10 Hz, 4 H). The doublets at 6·6 and 7·2 integrating to a total of 8 protons and forming a clear A_2B_2 pattern, are due to protons at I-3′, II–3′, I–5′, II–5′ and I–2′, II–2′, I–6′, II–6′ positions. This typical A_2B_2 pattern is usually observed when C–4′ is substituted. A comparison of the NMR spectrum of the diacetate (4) with those of the methyl ethers of biflavones[1,9–11] indicates that the singlet at 6·5 is due to the two equivalent protons at the I–6 and II–6 positions.



(1)



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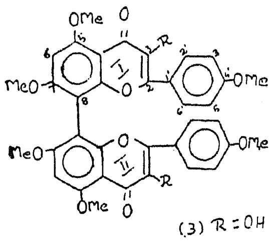

(3) R = OH

(4) R = $OCOCH_3$

The author gratefully acknowledges the helpful advice and discussion of Prof. R. D. Tiwari and Dr. (Mrs.) Krishna Misra. Thanks are also due to the Council of Scientific and Industrial Research, New Delhi, for financial assistance.

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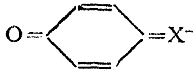
1. Murti, V. V. S., Raman, P. V. and Seshadri, T. R., *Tetrahedron*, 1967, **23**, 397.
2. Nakazawa, K. and Ito, M., *Bull. Chem. Pharm. Soc. (Japan)*, 1963, **11**, 283.
3. Sethna, S. and Mathai, K. P., *J. Indian Chem. Soc.*, 1964, **41**, 347.
4. Gandhi, P. and Tiwari, R. D., *Ibid.*, 1975, **52**, 1111.
5. Ahmad, S. and Razaq, S., *Tetrahedron Lett.*, 1971, 48, 4633.
6. Gandhi, P. and Tiwari, R. D., *Curr. Sci*. 1976, **45**, 18.
7. Algar, J. and Flynn, J. P., *Proc. R. Irish Acad.*, 1934, **42B**, 1.
8. Oyamada, T., *J. Chem. Soc. (Japan)*, 1932, **55**, 1256.
9. Batterham, T. S. and Highet, R. J., *Aust. J. Chem.*, 1964, **17**, 428.
10. Ilyas, M., Usmani, J. N., Bhatnagar, S. P., Ilyas, M., Rahman, W. and Pelter, A., *Tetrahedron Lett.*, 1968, p. 5515.
11. Pelter, A., Warren, P., Ilyas, M., Usmani, R. H. and Rahman, W., *Experientia*, 1969, **25**, 350.

ON THE MECHANISM OF THE ELBS PEROXYDISULPHATE OXIDATION

THE kinetics of the Elbs oxidation of phenols by peroxydisulphate in alkaline medium has been investigated by Behrman[1,2] and it has been concluded[2] that it is difficult to decide whether the rate limiting attack by the peroxydisulphate ion on the phenoxide is at the oxygen or carbon atom. Behrman also concluded that the evidence from the Hammet plots[2] is not of great value in distinguishing the above two possible electrophilic attacks. We wish to report here the possible attack by a careful analysis of the kinetic data.

In the case of *p*-halogenophenols, the rate of oxidation of *p*-fluorophenol is almost twice than that observed for phenol and the rates of oxidation of *p*-bromo- and *p*-iodophenols are lower. *p*-Chlorophenol has the same rate constant as that of phenol (Table I). The observed rate constants cannot be explained on the basis of + M effect of the halogen atoms though this effect is in the order *p*-F > *p* Cl > *p*-Br > *p* I. If this effect is important, then the rate constants of all the *p*-halogenophenols should be higher than phenol. A clue as to the possible site of attack as well as an explanation for the observed reactivities may be obtained if we take into account the possible *d*-orbital resonance in the case of *p*-chloro-, *p*-bromo and *p*-iodophenols. It has been well established by Baliah and co-workers[3-5] that chlorine, bromine and iodine can expand their valence shells by the utilization of their vacant *d*-orbitals. Such a *d*-orbital resonance becomes significant when electron-donating groups are present para to the halogen atom (Cl, Br, or I). In the Elbs oxidation, phenoxide ion is present and due to the *d*-orbital participation *p*-bromo- and *p*-iodophenoxides receive a significant contriubution from structure A, which will reduce the electron density at oxygen. In *p*-fluorophenoxide such a $d\pi$-bonding is not possible and due to the + M



A

X = Cl, Br or I

TABLE I

The effect of substituents on the rate of oxidation of phenol[a]

Phenol	k_2,1-/mol-min	Phenol	k_2,1/mol min.
Phenol	$1 \cdot 16 \pm 0 \cdot 00$	*m*-Fluorophenol	$0 \cdot 752 \pm 0 \cdot 003$
p-Fluorophenol	$2 \cdot 42 \pm 0 \cdot 01$	*m*-Chlorophenol	$0 \cdot 350 \pm 0 \cdot 004$
p-Chlorophenol	$1 \cdot 17 \pm 0 \cdot 04$	*m*-Bromophenol	$0 \cdot 383$
p-Bromophenol	$0 \cdot 970 \pm 0 \cdot 04$	*m*-Iodophenol	$0 \cdot 398$
p-Iodophenol	$1 \cdot 05 \pm 0 \cdot 00$	*m*-Methoxyphenol	$2 \cdot 55 \pm 0 \cdot 1$
p-Methoxyphenol	$20 \cdot 6 \pm 1 \cdot 0$	*m*-Methylphenol	$1 \cdot 44 \pm 0 \cdot 06$
p-Methylphenol	$5 \cdot 62 \pm 0 \cdot 05$	*m*-Cyanophenol	$0 \cdot 225 \pm 0 \cdot 003$
p-Cyanophenol	$0 \cdot 140$	*m*-Carboxyphenol	$0 \cdot 327 \pm 0 \cdot 009$
p-Carboxyphenol	$0 \cdot 155 \pm 0 \cdot 005$	2, 6-di-*t*-Butylphenol*	$31 \cdot 0 \pm 0 \cdot 5$

[a] General conditions; Phenol-peroxydisulphate ratio = 10; 1·7 M KOH Temperature 30°.

* In the case of 2, 6 di-*t*-butylphenol, 0·85 M KOH, 40% dioxane under nitrogen. All the values are taken from reference 2.

effect of the fluorine atom the electron density at oxygen will increase during the course of the reaction. The different reactivities of the *p*-halogenophenols now become understandable if the site of attack is oxygen and not the ortho-carbon atom. The rate constants for *p*-chloro-, *p*-bromo- and *p*-iodophenols are either equal to or lower than phenol since the *d*-orbital resonance reduces the electron density at oxygen. The singular behaviour of *p*-fluorophenol, that is its higher rate constant, results due to the + M effect of fluorine. If the above explanation is correct one should expect all the *m*-halogenophenols to behave in a similar manner since contributions from structure A is not possible. In fact the experimental results indicate that all the four *m*-halogenophenols have lower rate constants than phenol (*see* Table I).

If the attack is at oxygen one should expect that when an electron-releasing group is present in a para-substituted phenol then it will have a higher rate constant than phenol and the rate constant will be lower if the substituent is electron-withdrawing. The results in Table I substantiate this prediction. *p*-Methoxyphenol is oxidised about 20 times faster than phenol and *p*-cyanophenol about 10 times lower than phenol. It appears, therefore, that in the absence of steric effects the electrophilic attack is at oxygen.

The authors wish to express their thanks to Prof. N. Arumugam for helpful discussions.

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1. Behrman, E. J. and Walker, P. P., *J. Am. Chem. Soc.*, 1962, **84**, 3454.
2. —, *Ibid.*, 1963, **85**, 3478.
3. Baliah, V. and Uma, M., *Naturwissenschaften*, 1958, **21**, 512.
4. — and Srinivasan, C., *Indian J. Chem.*, 1971, **9**, 217.
5. Srinivasan, C., *Curr. Sci.*, 1975, **44**, 618.

1, 3-ADDITION REACTIONS OF BENZONITRILE OXIDES TO BENZIMIDAZOLES : SYNTHESIS OF 1-[(HYDROXYIMINO)ARYLMETHYL]-1*H*-BENZIMIDAZOLES AND THEIR 2-METHYL ANALOGS

PRIMARY and secondary amines undergo 1, 3-addition reactions with benzonitrile oxides to give the corresponding benzamide oximes[1,2]. Such reactions have not been investigated so far using heterocyclic bases containing N-H function. The 1, 3-addition of benzonitrile oxides with benzimidazoles have now been carried out to know the course of the reaction and the nature of the products formed.

When benzhydroximic acid chloride[3] (0·01 mole) was allowed to react with benzimidazole (0·02 mole) in methanol at room temperature, a colourless crystalline compound, m.p. 181° C ($M+237$) separated out from the reaction mixture. Its i.r. spectrum (KBr) showed an intense, broad absorption at 3100–2500 cm^{-1} (oxime O–H) and another absorption of medium intensity at 1600 cm^{-1} ($C=N$). On the basis of this evidence and analytical data, the compound has been assigned the 1-[(hydroxyimino) phenyl methyl]-1H-benzimidazole (I, R = H, Ar = Ph) structure. Similarly, 2-methyl-benzimidazole also reacted with benzhydroximic acid chloride to give I (R = CH_3, Ar = Ph). This reaction has been extended to differently substituted benzhydroximic acid chlorides and the products obtained have been characterised as I (Table I) by analogy. That the reaction is proceeding through the intermediacy of benzonitrile oxide is confirmed by reacting the latter, freshly generated from benzhydroximic acid chloride and triethylamine, with benzimidazole (R = H), when I (R = H, Ar = Ph) is obtained in almost quantitative yield.

TABLE I

1-[*Hydroxyimino) arylmethyl*]-1H-*benzimidazoles*, I[1]

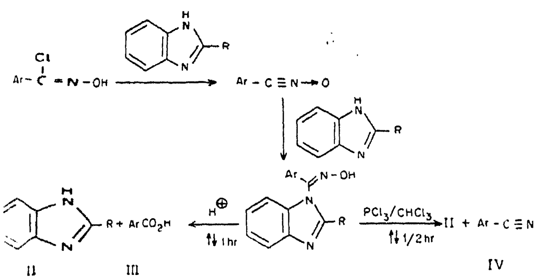
Sl. No.	Ar	R=H		R=CH_3	
		Yield (%)	M.P.[2] (°C)	yield (%)	M.P.[2] (°C)
1.	Phenyl	75	181	73	218
2.	4′-methylphenyl	87	201	80	210
3.	2′-chlorophenyl	70	196	88	221
4.	4′-chlorophenyl	72	199	72	233
5.	3′-nitrophenyl	96	221	70	231
6.	4′-nitrophenyl	75	227	72	247
7.	4′-methoxy-phenyl	78	210	..	..
8.	3′, 5′-dichloro-4′-methoxy-phenyl	75	221	..	..

1. All the compounds are recrystallised from methanol and gave satisfactory analytical values for carbon, hydrogen and nitrogen.
2. Melting points are uncorrected.

I (Ar = Ph, R = H) on acid hydrolysis under refluxing conditions resulted in the formation of benzimidazole (II, R = H) and the acid (III, Ar = Ph). Beckmann rearrangement of I using reagents like thionyl chloride, phosphorus pentoxide and polyphosphoric acid did not result in the formation of either of the two possible anilides.

Attempted dehydrogenative cyclisation reactions of I (Ar = Ph, R = H) to 3-phenyl-(1, 2, 4)-oxadiazolo-4, 5–a)-benzimidazole using reagents like alkaline otassium ferricyanide, ferric chloride were also unuccessful. However reaction of I (Ar = 3, 3′-dichloro-4′-methoxyphenyl, R = H) with phosphorus trichloide in chloroform gave II (R = H) and the nitrile V (Ar = 3, 3′-dichloro-4-methoxyphenyl). The reults are summarised in Chart I.

CHART – I



Full details of the dehydrogenative cyclisation and rolysis results of I will be published elsewhere.

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Werner, A., *Ber. dt. Chem. Ges.*, 1894, **27**, 2846.

Eloy, F. and Lenaers, R., *Chem. Rev.*, 1962, **62**, 155 and the references cited therein.

Perold, G. W., Styn, A. P. and Von Reiche, F. V. K., *J. Am. Chem. Soc.*, 1957, **79**, 462.

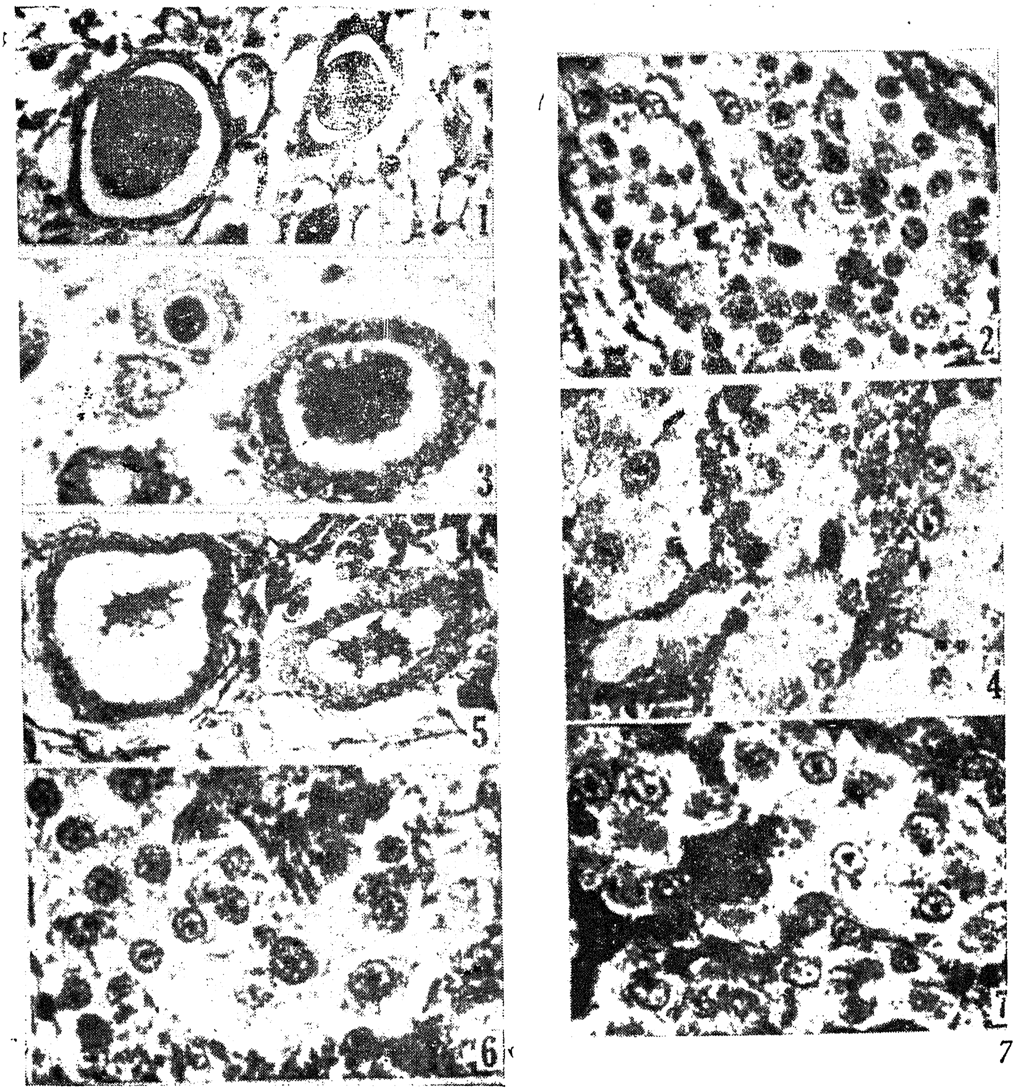
AMMONIUM SULPHATE AS A THYROID NHIBITOR IN THE FRESHWATER TELEOST *CLARIAS BATRACHUS* (L.)

ESENCE of pesticide residues has been reported in fishes by several investigators[3,9]. But very little known about the effect of commonly used fertilizers on the endocrine physiology of the fishes. As the thyroid gland of the teleosts is in the form of scattered follicles in the pharyngeal region, surgical thyroidectomy is not possible. Hence, thyroidectomy is accomplished with the aid of radioiodine[5,6]. Thyroid inhibitors like propylthiouracil and thiourea[2,4,10] are used to inhibit the secretion of thyroxine. It is known that when thyroxine level in the blood is reduced by the use of inhibitors or through radiothyroidectomy the thyrotrophs of the pituitary are stimulated to produce more of thyroid stimulating hormone (TSH). This feedback phenomenon is utilized to identify the thyrotrophs in the fishes[1,5–8,10].

60 *C. batrachus* used in this study were divided into three groups of twenty each, and, ten fish in each group were kept as controls. Group 1 was administered intraperitoneally 1·0 mCi of radioiodine (I-131) in four equal instalments of 250 μCi at three monthly intervals. Group 2 was kept in 0·01% ammonium sulphate for a year. Group 3 was maintained in 0·03% thiourea as used by several earlier investigators[2,7,10] for three months. The aquarium water containing these salts was replaced three times a week for groups 2 and 3. All of them were sacrificed by decapitation and their pharyngeal region with the thyroid and pituitary were fixed in Boun's fluid and Bouyin's sublimate respectively. The thyroid was stained in periodic acid Schiff (PAS) and haematoxylin. The pituitary was stained in PAS and lead haematoxylin, Herlant's tetrachrome and Alcian blue-PAS-Orange-G.

In *C. batrachus* the thyrotrophs are situated in the ventral aspect of the proximal pars distalis and in the adjacent neurohypophysial branches of the pars intermedia, comparable to that of *Heteropeneustes fossilis*[1]. In the ammonium sulphate treated fishes, the thyroid follicles exhibited hypertrophy and hyperplasia and reduction in their colloid content (Fig. 5) in comparison to the controls (Fig. 1). This compares well with those treated with thiourea (Fig. 3). The thyrotrophs of all the fishes treated with thiourea (Fig. 4), radioiodine (Fig. 6) and ammonium sulphate (Fig. 7) exhibited marked hypertrophy and degranulation. Their large rounded nuclei have prominent nucleoli. In the control fishes the thyrotrophs are small and the nuclei have clumps of chromatin material and lack prominent nucleoli (Fig. 2).

Thus, the histological picture of the thyroid and thyrotrophs of the fishes treated with known thyroid inhibitors are comparable with those exposed to ammonium sulphate. The thyroid inhibiting property of this commonly used fertilizer is apparently reported for the first time. Further studies on the radioactive iodine uptake and protein bound iodine (PBI) are in progress.



FIGS. 1–7. Fig. 1. Control thyroid follicles with flat epithelium and abundant colloid, × 360. Fig. 2. Showing thyrotrophs of a control fish, × 900. Fig. 3. Thiourea treated follicles with thick epithelium and reduced colloid, × 300. Fig. 4. Thiourea treated thyrotrophs exhibiting hypertrophy and degranulation, × 900. Fig. 5. Ammonium sulphate treated follicles showing thickening of epithelium and reduction of colloid, × 300. Fig. 6. Hypertrophying and degranulating thyrotrophs of I–131 treated fish, × 900. Fig. 7. Hypetrophied and degranulating thryotrophs of an ammonium sulphate treated fish, × 900.

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---

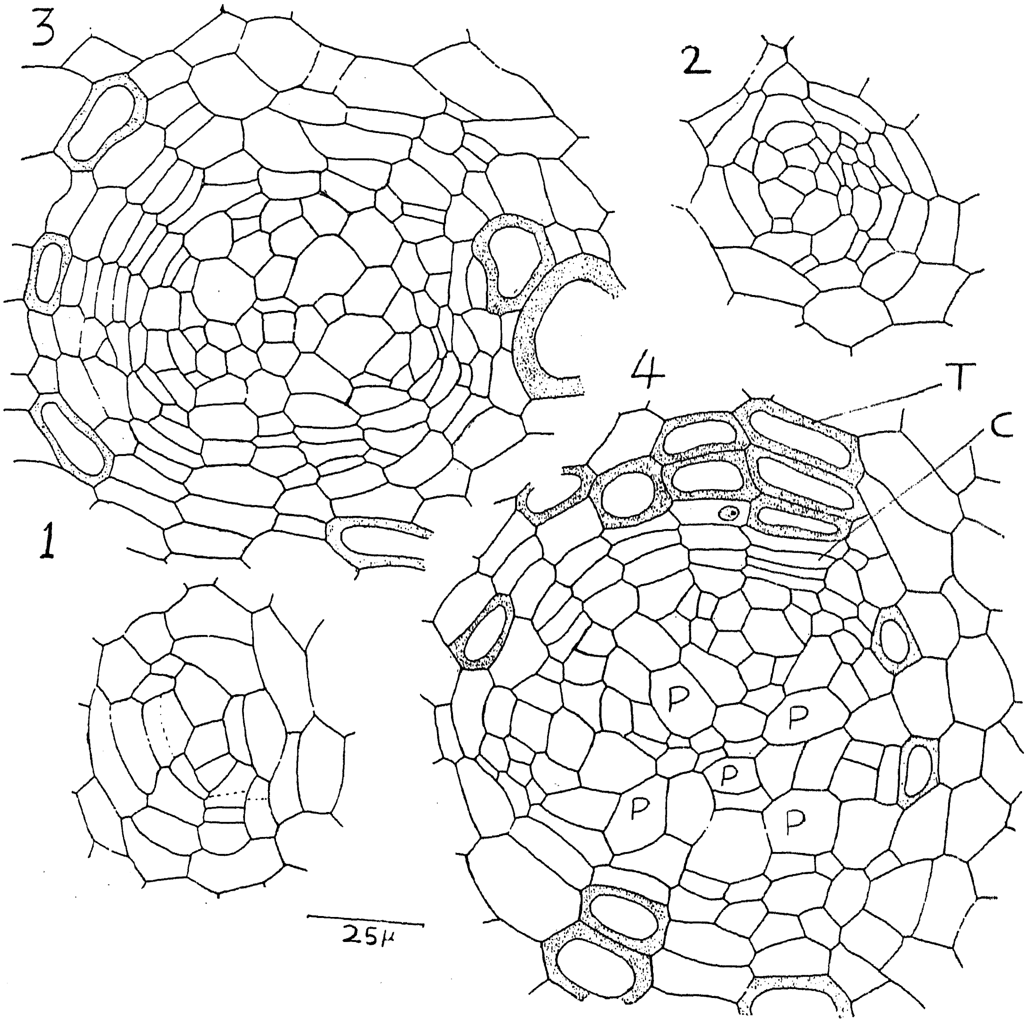
1. Baker, B. I., Keshavanath, P. and Sundararaj, B. I., *Cell Tiss. Research*, 1974, 148, 300.
2. Holmes, R. L. and Ball, J. N., *The Pituitary Gland*, Cambridge University Press, 1974.
3. Johnson, D. W., "Pesticide residues in fish," In *Environmental Pollution by Pesticides*, ed. Edwards, C. A., Plenum Press, New York, 1973, p. 182.

4. Leray, C. and Blanc, N., *Gen. Comp. Endocrinol.*, 1967, 9, 468.
5. Olivereau, M., *Ibid.*, 1963, 3, 312.
6. —, *Z. Zellforsch*, 1972, **128**, 175.
7. Sage, M., *Gen. Comp. Endocrinol.*, 1967, **8**, 314.
8. Shamim Haider, *Endocrinol.*, 1975, **65**, 300.
9. Shultz, C. D., Yauger, Jr. W. L. and Bevenue, A., *Bull. Envir., Contam. and Toxi.*, 1975, **13**, 206.
10. Srivastava, S. S. and Sathyanesan, A. G., *Acta Anat.*, 1971, **79**, 559.

VASCULAR NODULES IN THE STEM GALLS OF *COCCINIA INDICA*

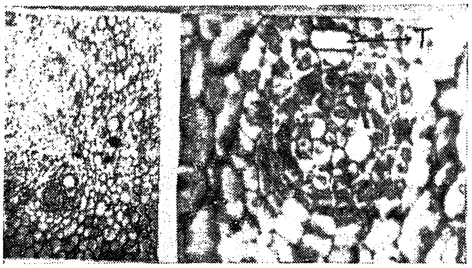
VASCULAR nodules analogous to those normally observed in callus obtained through tissue culture are encountered in the stem galls of *Coccinia indica* caused by the dipteran insect, *Neolasioptera cephalandrae*. These nodular islets are embedded in the reaction parenchyma of the galls. Since the occurrence of vascular nodules has not yet been reported in insect galls[1], the present contribution is made.

The larval entry causes the hypertrophy of the ground parenchyma of the stem that results in the galling. One to few cells of the reaction parenchyma form the initials for the nodules. These cells acquire deep cytoplasmic contents and divide in a periclinal or obliquely periclinal plane to result in a nest of cells (Fig. 1). To start with cell division is not followed by cell enlargement and so the cells derived are of much smaller in volume than their initial. After these initial divi-



FIGS. 1–4. Successive stages in the ontogeny of the vascular nodule. (C—Cambiform layers; P—Phloem; T—Tracheary elements.)

sions the meristematism is gradually restricted to the cell layers that are cambiform in transverse sections (Figs. 2–4). Cells inner to these regularly arranged layers as well as those that are derived from them on the inside differentiate as phloem while the outer derivatives mature *in situ* as tracheary elements. Thus, each nodule consists of a centrally located phloem, a few cambiform layers and the tracheary cells that surround them. The tracheary elements are very short, mostly perforate and are comparable in pitting and wall ornamentations to those normally encountered in the secondary xylem. The authors have made sure that bits of original procambial or cambial tissues are not involved in the organisation of these nodules.



5 6

FIGS. 5–6. Fig. 5. A portion of the transection of the gall showing the topography of the nodules. Fig. 6. A single nodule enlarged showing phloem cells (P) and Tracheids (T).

Gautheret[2] has summarized the available information on the vascular nodules (developed from callus cells) obtained in tissue cultures. He has recognized three types of nodules based on the relative disposition of xylem and phloem. In the first type the tissues are collateral, in the second, the phloem is surrounded by xylem and in the third, the xylem is enclosed by phloem. The vascular nodules present in Coccinia galls belong to the second category.

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Shillong, *August* 10, 1977.

---

1. Mani, M. S., *Ecology of Plant Galls*, The Hague, 1965.
2. Gautheret, R. J. In *Cell Differentiation and Morphogenesis*, p. 55.

DUAL ROLE OF THE TAPETUM

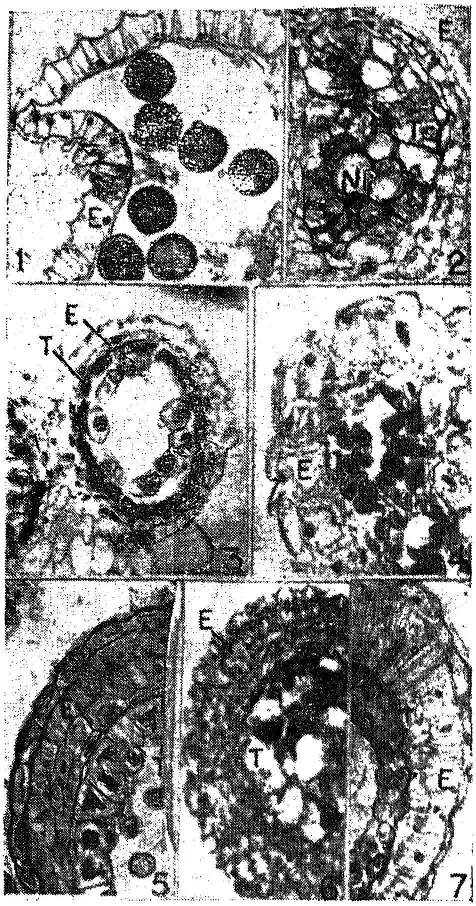
THE role of tapetum in the development of pollen grains has been established beyond doubt[1]. Little attention has been paid towards understanding the mode of endothecial development and its role in inducing male sterility. The present study has been undertaken to obtain the detailed information about the development of endothecium in male fertile plants and comparing it with their genic, cytoplasmic, gene-cytoplasmic and chemically induced male-sterile lines of *Allium cepa* L., *Beta vulgaris* L., *Capsicum annuum* L., *Cucumis melo* L., *Cucurbita maxima* Duch., *Datura alba* L., *Ranunculus muricatus* L., *Sesamum indicum* L. and *Triticum aestivum* L.

Endothecium is single-layered in the anthers of all the above-mentioned plants except *Sesamum indicum* where it becomes two layered at places. In the anthers of male fertile lines of these plants, the endothecial cells remain thin-walled and small from the time of their differentiation up to the early vacuolate microspore stage. During subsequent stages, these cells elongate radially and at late vacuolate pollen stage their enlargement reaches a peak. The characteristic fibrous thickenings appear on the radial walls of these cells (Fig. 1). This is accompanied by the degeneration of tapetal protoplast and by the time the thickenings develop within the endothecial cells, the tapetal protoplasts disintegrate completely.

These observations support the hypothesis that a product from the tapetum inhibits endothecial development of the anther and only after complete tapetal disintegration, inhibitor production ceases to occur and renders the normal development of endothecium possible[2].

This is also supported by the present observations on genic, cytoplasmic and gene-cytoplasmic male-sterile plants. The development of endothecium in most of the anthers of these sterile plants is completely inhibited (Figs. 2, 3). In such anthers, the tapetum does not show the usual cataclymic mode of development, but instead, shows diverse forms of abnormal behaviour[3]. It seems that the malfunctioning tapeta in the anthers of these male-sterile plants fail to provide nutrition to the developing microspores and consequently render them sterile. On the other hand, these tapeta continue to produce the inhibitor substance that checks the development of endothecium and finally makes the anther indehiscent. This is further supported by the fact that in a few anthers of CMS *Allium cepa* and *Capsicum annuum*, the endothecium develops normally but only after tapetum has degenerated completely (Fig. 4).

The programmed control of endothecium development by tapetum is further confirmed by the present findings on chemically treated plants. In certain treatments inducing partial sterility, the degeneration of tapetum is delayed. This is followed by normal endothecial development. In some other treatments causing either partial or complete sterility, the development of thickenings is fully inhibited (Figs. 5, 6). In such anthers, the tapetum is abnormal as is observed in genic or cytoplasmic male-sterile lines. In still other treatments, although cent per cent sterility is induced, the tapetal cells degenerate and facilitate endothecial development (Fig. 7). These observations are supported by the findings on *Belamcanda chinensis*[4] and CMS *Capsicum annuum*[5].



FIGS. 1–7, T.S. part of anthers of MF, GMS, CMS and IMS plants, × 160. Fig. 1. MF *Rannunculus muricatus* showing pollen grains, endothecial cells with characteristic fibrous bands and tapetum has degenerated. Fig. 2. GMS *Cucumis melo* showing hypertrophied tapetum and non-viable pollen grains. Fibrous bands absent. Fig. 3. CMS *Triticum aestivum* showing persistent tapetum and absence of fibrous bands. Fig. 4. CMS *Allium cepa* showing fibrous bands in endothecium and tapetum has degenerated. Fig. 5. IMS *Capsicum annuum* treated with 0·1% dalapon. Note intact tapetum, absence of fibrous bands and non-viable pollen grains. Fig. 6. IMS *Sesamum indicum* treated with 0·5% FW-450, showing hypertrophied tapetum and absence of bands. Pollen have degenerated. Fig. 7. IMS *Capsicum annuum* treated with 0·5% maleic hydrazide. Note the presence of fibrous bands, non-viable pollen grains and tapetum has degenerated. (E, Endothecium; NP, Non-viable pollen grains; T. Tapetum.)

The present study thus reveals that the tapetum not only controls pollen development but also plays an equally important role in the development of endothecium. The author is thankful to U.G.C., New Delhi, for financial assistance.

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---

1. Mascarenhas, J. P., *Bot. Rev.*, 1975, **41** (3), 259.
2. DeFossard, R. A., *Bot. Gaz.*, 1969, **130** (1), 10.
3. Chauhan, S. V. S., *Curr. Sci.*, 1976, **45** (7), 274.
4. Gupta, S. C. and Nanda, K., *Bot. Gaz.*, 1973, **134** (2), 125.
5. Novák, F., *Z. Pflanzenzucht*, 1971, **65**, 221.

INFLUENCE OF *AZOTOBACTER* ON GERMINATION OF RICE AND COTTON SEEDS

Azotobacter chroococcum is a soil inhabiting nitrogen fixing bacterium which is also known to secrete growth promoting factors as well as antifungal antibiotics[1,2,3,4]. By virtue of these attributes, pre-treatment of seeds with a suspension of *A. chroococcum* has generally shown improvement in seed germination and plant stands but experimental evidences in support of these observations are lacking. Therefore, experiments were done with rice (*Oryza sativa* var. IR 20) and cotton (*Gossypium* sp. var. JK 79) to quantify the influence of different strains of *A. chroococcum* on seed germination.

Seeds were surface sterilized and treated with 4 days old liquid cultures containing *A. chroococcum* (10^8 cells/ml). The control seeds were treated with the sterilized medium alone. They were sown on water soaked sterile filter paper in petridishes. The surface sterilant for rice seed was 0·1% $HgCl_2$ (3 minutes) and the sterilant for cotton seed was conc. H_2SO_4 (2 minutes) followed by successive washings with sterile distilled water.

In general, rice seeds responded better to *Azotobacter* inoculation than cotton seeds. Out of 18 strains of the bacterium, only 5 increased the germination significantly in the case of cotton. Two of the cultures

TABLE I

Effect of Azotobacter *strains on the germination of cotton seeds*

Cultures of *A. chroococcum*	Germination % (Mean of 60 seeds)	Cultures of *A. chroococcum*	Germination % (Mean of 60 seeds)
Control (without *Azotobacter*)	43·3	P_1	30·0
Bar 1	45·0	P_2	38·3
B_1	33·3	P_4	46·6
B_2	60·0	W_1	33·3
M_2	76·6	W_2	43·3
M_4	60·0	W_3	35·0
M_5	41·6	W_4	43·3
M_6	66·6	W_5	45·0
M_7	50·0	mp_3	26·6
M_8	55·0		
F value			Significant
C.D. at 5%			10·2

TABLE II

Effect of inoculation with Azotobacter *strains on the germination of rice seeds*

Strains of *A. chroococcum*	Germination (%)	(Mean of 60 seeds)	after
	48 hours	72 hours	120 hours
Control (without *Azotobacter*)	..	62·5	75·0
Bar 1	45·0	65·0	83·3
B_1	50·0	61·6	80·0
B_2	58·3	71·6	83·3
G_1	41·1	55·1	78·7
M_2	58·3	60·0	91·8
M_3	55·3	68·3	78·3
M_4	53·3	66·6	71·6
M_5	56·6	71·6	78·3
M_6	61·6	66·6	88·3
M_7	46·6	55·0	81·6
M_8	50·0	58·6	80·0
P_1	55·0	60·0	85·0
P_2	65·0	68·0	76·6
P_4	56·6	70·0	81·6
mp_1	..	51·6	73·3
mp_3	..	55·0	83·3
F value			Significant
C.D. at 5%			5·11

showed significant depressive effect on germination, although 6 of them showed general reduction in germination (Table I). In rice, 8 out of 16 strains improved the germination significantly and no depressive effect was seen (Table II). It is noteworthy that a strain (P_1), which stimulated the germination of rice, actually depressed the germination of cotton.

These results point out that careful choice of the strain of bacteria for individual hosts is an essential first step to achieve maximum benefit by *Azotobacter* inoculation.

However, stimulation of germination of seed by bacterization can be attributed safely to the production of growth promoting substances by this bacterium. On the other hand, the depressive effect of the bacterium on germination cannot be attributed to any known factor and further investigations in this regard are necessary.

The authors thank Dr. N. S. Subba Rao, Head of the Division of Microbiology for his guidance and help.

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1. Mishustin, E. N. and Shilnikova, V. K., *Rev. Research, Soil Biol.*, UNESCO, NRR IX, Paris, 1969, p. 79.
2. Shende, S. T., *Ph.D. Thesis*, Moscow, 1965.
3. Brown, M. E., Burlingham, S. K. and Jackson, R. M., *Plant and Soil*, 1964, 20 (2), 194.
4. Lakshmi-Kumari, M., Vijayalakshmi, K. and Subba Rao, N.S., *Phytopath. Z.*, 1972, **75**, 27.

ROLE OF SYNTHETIC DETERGENT ON THE GROWTH BEHAVIOUR OF *SPIRODELA POLYRHIZA* (L) SCHLEID

KALE[6] and Milwidsky[6] have described the general problem of pollution by synthetic detergent. It has been reported that 50% of the phosphorus in sewage effluent is derived from detergents in Sweden (Forsberg[2]) and in England (Owens and Wood[3]). The phosphorus in the detergent pollution comes from polyphosphate containing synthetic detergents.

So far little attention has been paid to assess the role which detergents play in the growth of aquatic vegetation. Evans and Robert[6] have reported that two species of *Potamogeton* (*P. pectinatus* and *P. densus*) were unable to grow in 2·5 mg/l concentration of alkyl benzene sulphonate (an important constituent of most of the synthetic detergents). Hynes[4] has shown that *Potamogeton densus* was killed in 14 days exposure to 6 mg/l of synthetic detergent (Ministry of Housing, London).

In India some work on the different aspects of detergent pollution has been done (Ghosh *et al.*[5], Mahajan *et al.*[6], and Gupta[7]). No attention, however, has been paid to assess its role on the growth of aquatic plants.

In the present study effect of various concentrations (2, 5, 10, 30, 50, 70, 100 and 200 mg/l) of a popular synthetic detergent powder 'Super Surf' has been studied on the growth of *Spirodela polyrhiza* (L) Schleid —a common duck weed found around Gorakhpur.

At each concentration, 1 g of the living plant material was inoculated and the growth behaviour was observed at 24 hr intervals for 15 days. The control set was kept in tap water. All the sets had 3 replicates. The chlorophyll content of 1 g of fresh plant was also estimated as per procedure recommended by Arnon[1].

It was observed that all the concentrations, above 50 mg/l of the detergent powder, were lethal to the plants. The maximum period, for which the plant survived, was 10 days in 70 mg/l and minimum 3-4 days in 200 mg/l.

When compared with the control set, it was observed that the chlorophyll content varied from 0·096 mg/g to 0·15 mg/g. It was maximum in 2 mg/l concentration and minimum in 50 mg/l concentration (Table I).

TABLE I

Chlorophyll content of Spirodela polyrhiza *(L) Schleid in different concentrations of detergent powder 'Super Surf'*

Concentration of Detergent Powder (mg/l)	Chlorophyll content of *Spirodela polyrhiza* (mg/g)
2·0	0·15
5·0	0·136
10·0	0·125
30·0	0·111
50·0	0·096
Control Tap water	0·144
Chlorophyll content of *Spirodela polyrhiza* before experimentation	0·140 mg/g

The constituents in 'Super Surf' (information obtained from manufacturer) were more or less similar to that of phosphorus containing synthetic detergents tested by Forsberg[2].

The phosphate in the detergent in low concentration seems to stimulate the growth of the *Spirodela polyrhiza*, as has also been observed by Forsberg[2], while assessing the role of detergents on the growth of algae. The death of plants was due to inhibitory effect of other components of detergent powder, like, alkyl benzene sulphonic acid and sodium salt.

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---

1. Arnon, D. I., *Plant Physiology*, 1949, p. 1.
2. Forsberg, C., *et al.*, *Sarhyck Urvatten Arg.*, 1967, p. 23.
3. Owens, M. and Wood, G., *Water Research*, 1968, **2**, 151.
4. Hynes, H. B. N., *The Biology of Polluted Water*, Liverpool University, 1971, p. 107.
5. Ghosh, B. B. *et al.*, *J. Inland Fishery Soc. Ind.*, 1973, p. 5.
6. *Proceedings of Symposium on Environmental Pollution. NEERI*, Nagpur, 1973, p. 9.
7. Gupta, S. K. *et al.*, *Indian J. Env. Health*, 1975, **17** (1), 21.

A NEW RECORD OF *KORDYANA* RAC. (EXOBASIDIACEAE) FROM MAHARASHTRA

DURING our recent mycological survey an interesting species of *Kordyana* Rac. (F. Exobasidiaceae) was collected from the Forests of Mahabaleshwar (Alt. 1360 mts) near Poona parasitizing leaves of *Commelina maculata* Edgew. The infection was characterised in the form of scattered irregular to subcircular chlorotic lesions turning deep-brown surrounded by yellow band at maturity. The fungus was found to grow profusely on these areas with the production of whitish powdery fungus growth consisting of tufts of basidia emerging through the stomata on the lower surface of the leaves. The fungus sporulated profusely on the host under conditions of cool and humid weather of monsoon (August–September). Both young as well as old leaves revealed typical infection equally well which in the advanced stages developed into necrotic lesions over-grown by the wooly fungus (Fig. 1A), No other part of the plant showed any infection. The fungus had the following morphological characters:

Hymenium semi-globose to globose, stromatic. Basidia external emerging in tufts bisterigmatic, long, cylindrical, hyaline, simple, measuring 22·8 30·4 × 3·8–4·7 μm. Basidiospores simple, hyaline, 1-celled, oblong to elliptical, measure 6·7–7·6 × 3·8–5·7 μm. Paraphyses numerous, flexuous and apically coiled (Fig. 1 B, C, D).

Diagnosis and Identity

A perusal of literature showed no report of any species of *Kordyana* on *Commelina maculata* Edgew. so far. The collection under study, on comparison, was found to differ greatly from *Kordyana indica* Gaum., in possessing short basidia and smaller basidiospores. Besides, it also proved to be distinct from *Kordyana celebensis* Gaum., as the latter is characterized by complete absence of paraphyses. However, the present fungus agreed in general morphology with *Kordyana polliae* Gaum.[2] (affecting *Pollia sorzogonensis* Endl. a tropical species of Commelinaceae) except for the size of basidiospores which are significantly smaller in the present collection as compared to *Kordyana polliae* (basidiospores 15–21 × 5–8 μm). Hence, the fungus under study has been identified as *Kordyana polliae* var. *microspora*, var. nov.

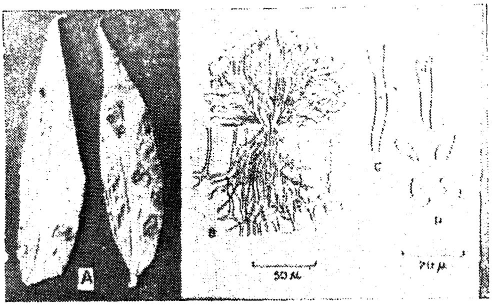

FIG. 1. *Kordyana polliae* var. *microspora* on *Commelina maculata*. A. Infection spots on leaves (Habit). B, C and D. Morphology of the fungus showing hymenium, basidia, and basidiospores.

So far, there are only three records of *Kordyana* spp. from India, *viz.*, *Kordyana colebensis* Gaum. on *Commelina attenuata* Koen., *Kordyana indica* Gaum. on *C. benghalensis* L[1]. and *Kordyana boswelliae* Thirum. Patel and Dhande on *Bowellia* sp[4]. Hence this is the first report of *Kordyana polliae* var. *microspora on Commelina maculata* Edgew. from India[3].

The material has been pdeosited at M.A.C.S., Poona under No. AMH 2186.

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---

1. Butler, E. J. and Bisby, G. R., *The Fungi of India*, I.C.A.R., New Delhi, 1960, p. 148 (Revised by R. S. Vasudeva).
2. Gaumann, E. A., *Ann. Mycol.*, 1922, 20, 257.
3. Mukerji, K. G. and Juneja, R. C., *Fungi of India*, Emkay Publ., Delhi, 1975, p. 224.
4. Vasudeva, R. S., *Fungi of India*, Suppl. I, I.C.A.R., New Delhi, 1962.

INDUCED TETRAPLOIDY IN MEDICINAL YAM *DIOSCOREA FLORIBUNDA* MART. AND GAL.

Dioscorea floribunda Mart. and Gal. is a new world medicinal yam cultivated for the alkaloid, diosgenin. This forms an ideal material for improvement through polyploidy, since it is propagated vegetatively and the economical product is the underground tuber. The genome of this species ($n = 18$)[1] is doubled through colchicine and a comparison of the diploid and tetraploid is given in this report.

Seeds were germinated in petri dishes and the seedlings were treated with 0·25% aqueous colchicine solution, when the seedlings were about 2″ in length. Seedlings were kept in petri dishes containing colchicine solution with only their root system dipping under the solution. The treatment was given for 20 hours. The seedlings were washed for 8 hours and sown in plastic bags. Three month old seedlings were transplanted in the field. Out of 10 surviving seedlings, 4 were females and 6 were males. Among the males, one was found to be a complete tetraploid and the other had several tetraploid chimeras. Chromosome number ($4n = 72$) was verified in acetocarmine smears.

Morphologically, the autotetraploid is more robust than the diploid with larger and thicker leaves, longer petioles, larger stomata, more number of shoots, larger stem diameter, longer internodes, more number of flowers per inflorescence and larger pollen grains (Table I, Fig. 1),

TABLE I

Morphological comparison of the induced tetraploid with its parent

Sl. No.	Character	Tetraploid	Diploid
1.	Leaf area (cm)^2	31·20	23·28
2.	Leaf thickness (μ)	22·66	18·80
3.	Stomatal length (μ)	3·50	2·00
4.	Petiole length (cm)	2·82	2·12
5.	Stem diameter (cm)	0·10	0·07
6.	Internode length (cm)	5·60	3·70
7.	Number of inflorescences	35·00	30·00
8.	Mean number of flowers per inflorescence	67·00	57·4
9.	Pollen diameter (μ)	3·60	2·33

Chromosome configurations ranging from univalents to quadrivalents occurred in the tetraploid in varying frequencies (Fig 2) but with a predominance of bivalents and quadrivalents (Table II). The diploid parent has regular meiosis with 18 bivalents. In spite of the presence of univalents and multivalents, meiosis was surprisingly regular in the induced polyploid.

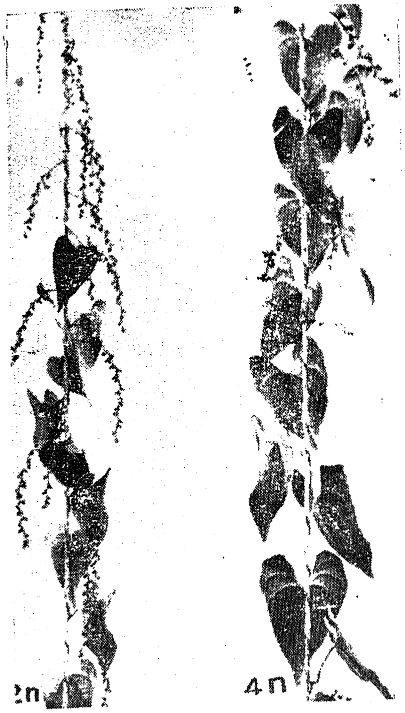

FIG. 1. Diploid (2*n*) and induced tetraploid (4*n*).

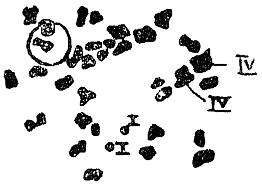

FIG. 2. Camera lucida drawing of a PMC at diakinesis in the induced tetraploid (I: univalent; IV: quadri valent).

TABLE II

Chromosome configurations in the induced tetraploid

	Chromosome configurations			
	I	II	III	IV
Range	0–6	18–36	0–2	0–9
Total	38	1280	14	240
Mean/Cell	0·76	25·6	0·28	4·8

No laggards could be observed at any stage in spite of the presence of univalents at diakinesis. This may be due to a genetic control of the orientation of the univalents resulting in their regular disjunction. Pollen tetrads were normal and pollen fertility is the same as that of the diploid (80–100%). The practical utility of the induced tetraploid is under study.

The author is thankful to Dr. G. S. Randhawa, Director, Indian Institute of Horticultural Research, for providing facilities and for his interest and encouragement.

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1 Rao, V. R. and Murty, U. R., *Curr. Sci.*, 1975, 44, 24.

A NEW SPECIES OF *TERMITOMYCES* FROM INDIA

DURING the summer of 1976 a species of *Termitomyces* was collected in the lawns of the Regional Research Laboratory, Jammu and it is described here as a new species. Colour terminology used is that of 'Methuen Handbook of Colour'[1].

Termitomyces radicatus Natarajan sp. nov (Fig. 1 *a*–*d*).

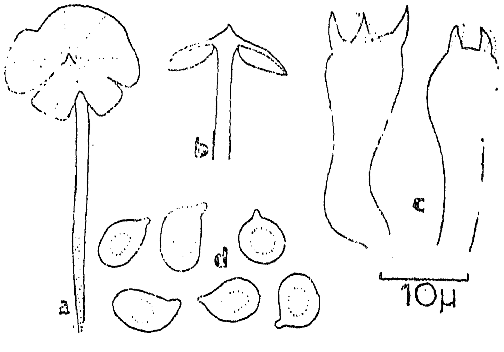

FIG. 1. *a*, Habit, × 2; *b*, Section, × 2; *c*, Basidia; *d*, Basidiospores.

Pileus ad 3·5 cm diametro, primo convexus, postea planconvexus, postremo vero planus, perforatorio spiniformo prominente, superficie crocceo-alba (5A2), maturescente vero croceo-cinerea, laevia. Centrum pilei apud perforatorium vandyke brunneum (6F6). Superficies pilei epicutis hyphis repentibus, 1·5–4·0 μ latis, radiatim constantibus. Contextus ab epicute, strato hypharum hyalinarum inflatarum tenuidermarum ad 37·5 μ, separabilis. Contextus succulentus, albus, ex hyphis tenuidermarum intextarum constantibus.

Lamellae discretae, albae, erubescentae, ad 4 mm latae. Lamellulae adsunt. Stipes ad 4·5 cm longus, uniformiter crassus, glaber, luteo-albus (4A2), ad croceo-albus (5A2), cylindraceus, solidus, sine annulo. Psuedorrhiza adsunt, ad 2·5 cm longa. Trama hymenophoralis regulares, hyphis tenuidermarum parallelarum ad septa constrictarum, constantibus. Basidia 20·0–22·0 × 6·0–9·0 μ, clavata, 4 sterigmata ferentia. Et cheilocystidia et pleurocystidia nulla. Sporae 6·0–9·0 × 4·0 – 5·0 μ, ellipsoideae, laeves, submicroscopio hyalinae, non amyloideae. Fibulae nullae.

In terra, in solo argillaceo, prato Regional Research Laboratory, Jammu, 7 August 1976. Coll. K. Natarajan.

Pileus up to 3·5 cm in diameter, convex, becoming plano convex to plane with a prominent spineform perforatorium, surface orange white (5A2) becoming orange grey (5B2) when mature, smooth. The centre of the pileus in the region of perforatorium vandyke brown (6F6) in colour. Pileal surface an epicutis, consisting of radially arranged repent hyphae 1·5–4·0 μ wide. The context is separable from the epicutis by a layer of inflated thin walled hyaline hyphae which are up to 37·5 μ. Context fleshy, white, consisting of thin walled interwoven hyphae. Lamellae free, white, becoming pink, up to 4 mm broad. Lamellulae present. Stipe up to 4·5 cm long and 5 mm broad, uniformly thick, glabrous, yellow white (4A2) to orange white (5A2), cylindric, solid and without annulus. Pseudorhiza present, up to 2·5 cm long. Hymenophoral trama regular, consisting of thin walled parallel hyphae, constricted at septa. Basidia 20·0–22·0 × 6·0–9·0 μ, clavate, bearing 4 sterigmata. Both cheilocystidia and pleurocystidia absent. Spores 6·0–9·0 × 4·0–5·0 μ, ellipsoid, smooth, hyaline under the microscope, non-amyloid, clamp connections absent.

On ground, clayey soil, Lawn of Regional Research Laboratory, Jammu, 7th August, 1976. Coll. K. Natarajan, Herb. MUBL No. 2353.

The presence of a dark coloured spiniform perforatorium and a short pseudorhiza and absence of pleurocystidia and cheilocystidia differentiate this species from other species of the subgenus Praetermitomyces like *T. microcarpus*,[2] *T. medius*[2], *T. orientalis*[3], *T. narobiensis*[4], *T. tyleriana*[4], *T. badius*[5] and *T. indicus*[6].

This species which occur in abundant quantities is edible.

Thanks are due to Rev. Fr. K. M. Matthew S.J., St. Joseph's College, Tiruchirapalli, for correcting the Latin diagnosis and to Dr. E. Horak, ETH, Institut für Spezielle Botanik, Zürich, for his valuable comments.

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---

1. Kornerup, A. and Wanscher, J. H., *Methuen Handbook of Colour*, Methuen and Co., Ltd., London, 1967, p. 243.
2. Heim, R., *Arch. Mus. nat. Hist. nat. ser* 6, 1941, **18**, 107.
3. —, *Bull. Jard. Bot. Brux.*, 1951, **21**, 205.
4. Otieno, N. C., *Proc. E. Afr. Acad.*, 1964, **2**, 108.
5. —, *Sydowia*, 1969, **22**, 161.
6. Natarajan, K., *Kavaka*, 1975, **3**, 63.

THE EXOCELLULAR UREASE IN RICE ROOTS

SEVERAL workers[1,3-5] have reported the occurrence of free exocellular enzymes in soils which can solubilize macromolecular constituents of organic soils permitting uptake and utilization of the resultant low molecular weight organic compounds by the plants. But the exocellular accumulation and nature of urease (EC–3.5.1.5) in the rice root tips has not so far been reported. The present work deals with the exocellular accumulation of urease, in 5 high yielding varieties of rice. The distinction has been made between (A) urease apparently bound to the root surface, (B) exocellular urease released into the culture medium.

Improved varieties of rice (*Oryza sativa* L. var. Ratna, Kaberi, CO–13, TN–1 and MTU–17) were soaked in water for 12 hrs followed by surface sterilization with a soak in 0·1% mercuric chloride solution for 15 minutes and finally washed several times with sterile distilled water. The soaked seeds were germinated in a sterile moist chamber at room temperature and used at an age of 4 to 5 days when the roots of the seedlings were *ca.* 50 mm in length. The inorganic nutrient solution was prepared as described by Yoshida *et al.*[6]

Two incubation methods were followed[1]. For method (A), 8 intact seedlings each *ca.* 50 mm in length, were incubated at 27° C with 2 ml of 10% urea and 13 ml of inorganic nutrient solution. At 3, 6, 9, 12 hours intervals one ml of the reaction mixture was removed and the amount of urea hydrolysed was determined by the standard method[2]. In the second method (B) the eight seedlings were suspended in tubes containing 13 ml of inorganic nutrient solution. Samples of 1 ml of the nutrient were removed from the tubes at 3, 6, 9, 12 hours intervals and incubated with 2 ml of 10% urea solution. Rates of the reaction were determined as per method (A) above.

One urease number is defined as that amount of enzyme which liberates 1 mg of nitrogen as ammonia from urea under the assay conditions described above. Multiplication of the urease No. by 0·32 is expressed as the urease unit.

The roots of intact rice seedlings were tested for heir ability to hydrolyse urea. Table 1 presents rease activities as determined by both the

TABLE I

(A) Exocellular enzymes bound to root surface and (B) Exocellular enzymes released to nutrient solution. Data expressed as urease units

Variety	Method	Incubation period (hrs.)			
		3	6	9	12
atna	A	7·36	13·76	19·84	26·24
	B	0·19	0·4	0·64	0·83
O–13	A	6·72	12·8	18·88	25·6
	B	0·16	0·32	0·51	0·67
aberi	A	3·20	6·40	9·28	12·48
	B	..	0·16	0·3	0·44
–1	A	4·48	8·64	13·4	17·6
	B	..	0·13	0·2	0·27
TU–17	A	2·88	6·08	8·96	11·52
	B	..	..	0·09	0·14

thods A and B. As can be seen urease activity ild be detected when the roots of the seedlings e in contact with the substrate (Method A). w urease activity was also found in the root ture medium (method B). The reaction rate urease activity, as measured by procedure A, essentially a linear function of time. In the of activities measured by procedure B, the of release of enzyme into the nutrient immers- the roots, is again almost a linear function of e. The urease activity is high in varieties na and CO–13, medium in varieties Kaberi and –1 and low in MTU–17.

hese results suggest that exocellular urease ciated with the rice roots may hydrolyze organic substances independent of soil microbial ity.

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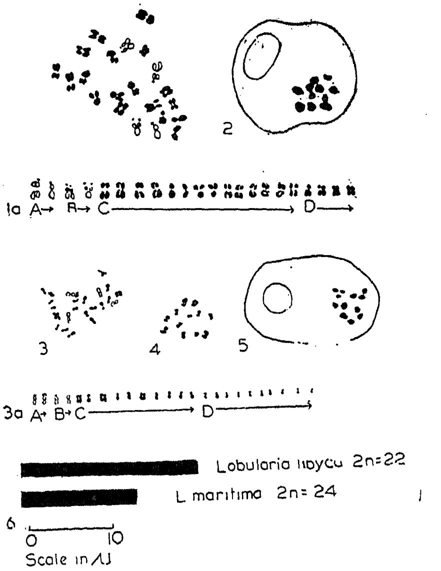
1. Chong Wchang, and Bandurski, R. S., *Pl. Physiol.*, 1964, 39 (1); 60.
2. Hofmann, E., *Methods of Enzymatic Analysis*, Ed., Bergmeyer, H. U., Academic Press, New York, 1963, p. 722.
3. Kivilaan, A., Beaman, T. C. and Bandurski, R. S., *Pl. Physiol.*, 1961, 33, 349.
4. Mouraret, M., *Mem. Orstom. Office. Res. Sci. Tech. Qustre–Mer.*, 1965, 9, 19.
5. Paulson, K. N. and Kurtz, L. T., *Soil. Sci. Soc. Amer. Proc.*, 1969, 33, 897.
6. Yosida, S., Forno, D. A. and Cock, J. H., *Laboratory Manual for Physiological Studies of Rice*, IRRI, 1971, p. 53.

CHROMOSOME ANALYSIS OF TWO SPECIES OF *LOBULARIA* (CRUCIFERAE)

Lobularia belongs to the tribe Alysseae of Cruciferae[7]. Two species of the genus, namely, *L. libyca* (Viv.) Meisn. and *L. maritima* (L.) Desv. were studied for an analysis of karyotype details.



FIGS. 1–6. Figs. 1, 1 *a* and 2. *Lobularia libyca*, 1 and 1 *a*, Somatic metaphase with $2n = 22$ chromosomes and its idiogram. 2, Metaphase II of meiosis showing 11 chromosomes in a nucleus. Figs. 3, 3 *a*, 4 and 5. *Lobularia maritima*. 3 and 3 *a*, Somatic metaphase with $2n = 24$ chromosomes and its idiogram. 4 and 5, Metaphase I and II showing 12 bivalents and 12 chromosomes respectively. Fig. 6. Histogram showing the total amount of chromatin matter in length of the haploid complements of the two species of *Lboularia*.

Materials and Methods:

Seeds of the two species were obtained through the courtesy of Hortus Botanicus Hauniensis,

TABLE I

Determination of the centromeric position by the centromeric index or F%

$$\left(F\% = \frac{\text{Short arm length}}{\text{Total length}} \times 100\right)$$

F%	50	37·5–49·9	25·1–37·4	25	18·6–24·9	12·6–18·5
Centromeric position	median	nearly median	nearly sub-median	sub-median	nearly sub-median	nearly sub-terminal

TABLE II

L. libyca (Viv.) Meisn.

$2n = 22 = 2A + 2B + 14C + 4D = 2{\cdot}5\text{–}1{\cdot}5\ \mu$ (Figs. 1, 1 *a*).

Chromosome type	No. of pairs	Length in μ	Short arm length in μ	F%	Nature of primary constriction	Remarks
A	1	2·5	0·41	16·6 50		Two constrictions, one median and the other nearly subterminal at the distal end of one arm.
B	1	2·08	0·83	50	median	Satellite at the distal end of an arm
C	3	2·08	0·83	40	nearly median	
	1	1·87	0·62	33·3	nearly median	
	3	1·6	0·52	31·2	nearly submedian	
D	2	1·5	0·41	26·6	nearly submedian	

Meiosis reveals the presence of 11 chromosomes at the second division of metaphase (Fig. 2).

Copenhagen, Denmark and of *Lobularia maritima* also from Suttons and Sons Pvt. Ltd., Calcutta. The seeds were germinated and the seedlings grown till maturity.

For meiosis, propiono-carmine method was followed. For somatic study, pretreatment with saturated solution of monobromonapthalene at 12–14° C for 1 hour to hour, 15 minutes followed by the usual propiono-orcein schedule gave well scattered metaphase plates. The figures were drawn at a table magnification of approximately × 2,400. In the somatic figures, the chromosomes with secondary constrictions were drawn in outline only. To locate the centromeric position, the centromeric index (F%) was determined for each chromosome pair (Table I).

Observation :

Detailed karyotype analysis for *Lobularia libyca* and *L. maritima* is given in Tables II and III respectively.

Discussion :

In the present study, $2n = 22$ and $n = 11$ chromosomes have been observed in the somatic and meiotic nuclei respectively in *Lobularia libyca*. *L. maritima* has $2n = 24$ chromosomes in the somatic cells[1-6]. This is extremely significant indicating that a particular chromsome number has become fixed

TABLE III

L. maritima (L.) Desv.

$2n = 24 = 2A + 2B + 10C + 10D = 1\cdot25\text{–}0\cdot83\,\mu$ (Figs. 3, 3 *a*).

Chromosome type	No. of pairs	Length in μ	Short arm length in μ	F%	Nature of primary constriction	Remarks
A	1	1·25	0·62 0·205	50 16·6		Primary and secondary constrictions, one median and the other nearly subterminal at the distal end of an arm.
B	1	1·25	0·62	50		Satellite at the distal end of an arm.
C	5	1·25–1·04	0·41	33·3–40	nearly submedian to nearly median	
D	5	0·83	0·205	25	submedian	

12 bivalents are present in metaphase I stage in meiosis and 12 chromosomes in each nucleous in metaphase II (Figs. 4, 5).

for the species concerned. In general the chromosomes of *L. libyca* are slightly longer than those of *L. maritima* and minute differences in chromosome morphology are quite evident. The cytological study of the other species of *Lobularia* from different populations is necessary to work out the role of cryptic structural changes of chromosomes in the evolution of species. In spite of the structural changes, regular bivalent formation is observed in the two species indicating homozygosity for these changes through selection.

The author wishes to express her indebtedness to Prof. A. K. Sharma, Ghosh Professor and Head, Department of Botany, University of Calcutta, for facilities provided and the University Grants Commission (Special Assistance Programme) for the award of a junior fellowship during the tenure of this investigation.

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1. Bjorkqvist, I., Von Bothmer, R., Nilsson, O. and Nordenstam, B., *Bot. Not.*, 1969, 122, 271.
2. Bonnet, A. L. M., *Nat. Monospel. Ser. Bot.*, 1963, 15, 41.
3. Gadella, Th. W. J. and Kliphuis, E., *Acta Bot. Neerl.*, 1966, 15, 484.
4. Jaretzky, R., *Jahrb. Wissensch. Bot.*, 1928, 68, 1.
5. Kliphuis, E. and Wieffering, J. H., *Acta Bot. Neerl.*, 1972, 21, 5.
6. Manton, I., *Ann. Bot.*, 1932, 46, 509.
7. Schulz, O. E., *Die natürlichen Pflanzenfamilien*, 2nd Edition, 1936, 17 B, 227.

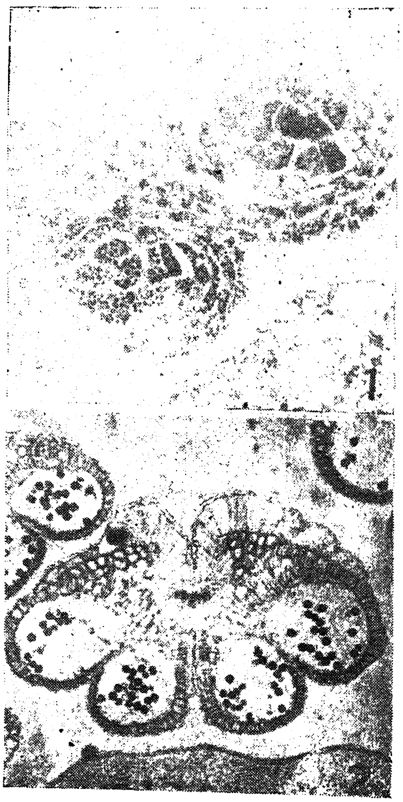
ANTHER IN *CAPPARIS DECIDUA* (FORSK.) PAX.

DURING the course of an investigation on floral morphology of some *Capparaceae*, the authors observed certain features of difference from the earlier description of the anther in *Capparis decidua* (Forsk.) Pax given by Narayana[2].

In each lobe of the young anther the archesporium differentiates as a plate of 4 to 6 cells. These undergo periclinal divisions cutting off the primary parietal layer, and primary sporogenous layer. The former undergoes further periclinal divisions so that four subepidermal layers are formed (Fig. 1). This is contrary to the earlier description of 5 to 7 layers reported by Narayana[2]. The development of the anther wall, therefore, follows the basic type[1].

The subepidermal layers subsequently differentiate as the fibrous endothecium, two ephemeral middle layers and secretory tapetum (Fig. 1). In our material the tapetal cells did not show any 'elaboration' as observed by Narayana[2] and remained 1-nucleate throughout as against his report of 2 or 3-nucleate condition. Concurrent with the close of the meiotic divisions in microspore mother cells, the tapetal cells show signs of degeneration and, finally, become completely emaciated as the anther matures (Fig. 2). The only wall layer that persists in dehiscing anther is the endothecium, though the crushed remains of the middle layers as also the degenerated tapetum can still be discerned (Fig. 2).

At the 1-nucleate stage of pollen, the endothecial cells as well as cells of the connective-side develop radially-disposed fibrous bands (Fig. 2), a situation that has been reported only in *Capparis grandis*[3]. The above report can thus be reckoned as the first one for *Capparis, decidua*. The epidermis persists, its cells enlarge, and radially elongate on the dorsal side of the anther, while those lining the pollen sac get flattened (Fig. 2); this is in conformity with the findings of Narayana[2].



FIGS. 1–2. Anther in *Capparis decidua* (Forsk.) Pax. Fig. 1. Transection of anther lobes showing wall layers, and sporogenous cells, × 360; Fig. 2. Same, showing 4-sporangiate condition, endothecium and the cells of the connective side have radially-disposed thickenings, remains of tapetum and sterile and fertile pollen grains, × 180.

Meiotic divisions in the pollen mother cells are regular, followed by simultaneous cytokinesis resulting in tetrahedrally-arranged pollen tetrads, occasionally decussate. The pollen grains are spheroidal, and 3-colporate with a thick, smooth exine, and a thin intine. They are shed at the 2-celled stage, and are packed with starch grains which mask the nuclei. There is about 35% of pollen sterility, a feature not recorded by Narayana[2].

One of us (B. H. Rao) gratefully acknowledges the award of a Junior Research Fellowship by the C.S.I.R., New Delhi. Thanks are expressed to Dr. T. A. Sarma, for providing the pickeled material, to Dr. A. S. Murty, for taking photomicrographs and to Professor A. S. Rao, for facilities.

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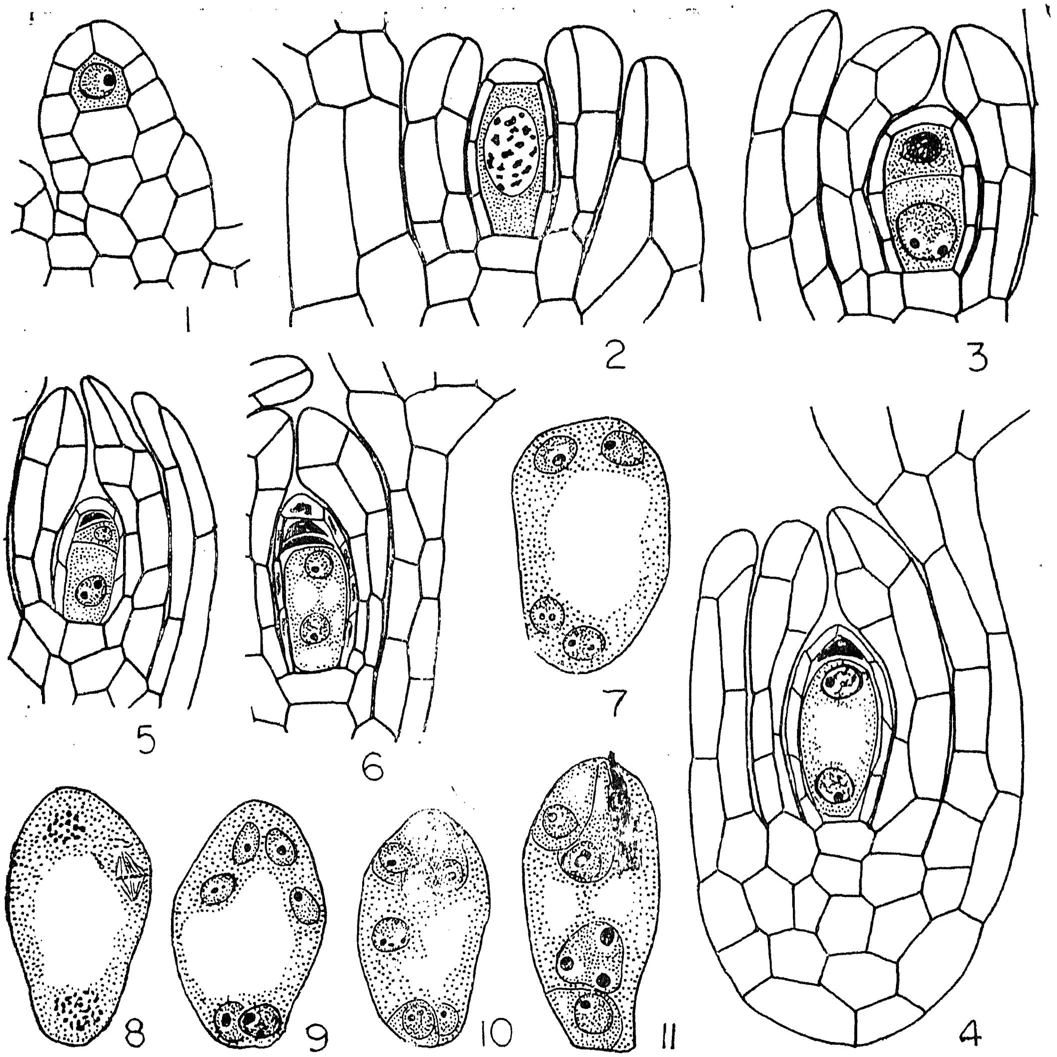
1. Davis, G. L., *Systematic Embryology of the Angiosperms*, John Wiley and Sons, New York, 1966.
2. Narayana, H. S., *Phytomorphology*, 1962, 12, 167.
3. Padhye, M. D. and Vaidya, P. B., *Curr. Sci.*, 1970, 39, 377.

ONTOGENY AND ORGANISATION OF FEMALE GAMETOPHYTE IN *POLYSTACHYA FLAVESCENS* (BL.) J. J. SMITH—A REINVESTIGATION

SWAMINATHAN[1] reported the occurrence of a bisporic, usually 8-nucleate and rarely 7-nucleate embryo sac in *Polystachya flavescens*. A reinvestigation of this aspect of the same taxon, collected near Devihalli, Sakaleshpur Taluk, Karnataka State, has revealed some interesting deviations which are presented below.

The ovular primordia are initiated after pollination. Each ovular primordium is composed of an axial row of 5–6 cells ensheathed by a single layer of epidermis. The uppermost cell of the row acts as the archesporial cell and it directly functions as the megaspore mother cell (Figs. 1–2). It undergoes meiosis I producing the dyad cells (Figs. 2–3). The upper dyad cell promptly degenerates while the lower functions and its nucleus undergoes meiosis II. The two resulting nuclei, then, become separated by a central vacuole. A bisporic 2-nucleate embryo sac thus results (Fig. 4). Occasionally, however, the lower dyad cell gives rise to 2 megaspores following meisosis II when a triad is observed (Fig. 5). The upper of the two megaspores degenerates while the lower functions and produces a 2-nucleate embryo sac (Fig. 6). In both bi- and monosporic embryo sacs so produced, the two nuclei undergo a simultaneous division leading to the formation of a 4-nucleate embryo sac (Fig. 7). The four nuclei of the sac begin their synchronous division. The two micropylar nuclei complete the division producing 4 small haploid nuclei while the spindles of the chalazal ones fuse together, ultimately giving rise to two large diploid nuclei (Figs. 8–9). As a result, a 6-nucleate embryo sac with 4 haploid nuclei at the micropylar part and two diploid nuclei at the chalazal end is formed. The micropylar quartet organises the egg apparatus consisting of an egg, two synergids, and a micropylar polar nucleus. Of the two diploid nuclei, one functions as the chalazal polar nucleus and the other contributes to the formation of a single antipodal cell (Fig. 10). The micropylar polar moves downwards and fuses with the chalazal polar producing a triploid secondary nucleus (Fig. 11). The mature embryo sac, therefore, contains an egg apparatus, a central cell and an antipodal cell.

Double fertilisation occurs. The tetraploid primary endosperm nucleus is usually located immediately above the antipodal cell (Fig. 11).



FIGS. 1–11. Fig. 1. An ovular primordium with archesporial initial. Fig. 2. MMC at prophase I. Fig. 3. yad cells. Fig. 4. 2-nucleate embryo sac (bisporic). Fig. 5. Triad. Fig. 6. 2-nucleate embryo sac (mono-oric). Fig. 7. 4-nucleate embryo sac. Fig. 8. 4–8 nuclear division stage. Note the fused spindles at e lower end. Fig. 9. 6-nucleate embryo sac. Fig. 10. Organised embryo sac. Fig. 11. Embryo sac on after fertilisation. (All figures, × 600).

The foregoing, therefore, reveals that the development of the female gametophyte in *Polystachya flavescens* is not only bisporic as recorded by Swaminathan[1], but also monosporic. Further, it is always, six-nucleate, the reduction in the number of nuclei at the chalazal end being due to the fusion of spindles at the 4-8 nuclear division stage of the embryo sac. Apparently, it is never 8-nucleate or 7-nucleate as reported by Swaminathan. Moreover, the fusion of polars occurs near the antipodal cell and not near the egg as stated by him (Swaminathan[1]).

The fusion of spindles during the last nuclear division leading to the formation of a 6-nucleate embryo sac with 4 haploid nuclei at the micropylar end and 2 diploid nuclei at the chalazal end has been reported as only occasional in *Epipactis pubescens* (Brown and Sharp[2]) and *Paphiopedilum insigne* (Afzelius[3]). However, it is a regular feature in *Polystachya flavescens.* This type of female gametophyte organisation essentially conforms to the G3[1] type of Abe[4].

One of the authors (KGE) is grateful to C.S.I.R. India, for the award of a Junior Research Fellowship.

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---

1. Swaminathan, D., *Curr. Sci.*, 1967, **36** (14), 384.
2. Brown, W. H. and Sharp, L. W., *Bot. Gaz.*, 1911, **52**, 439.
3. Afzelius, K., *Svensk Bot. Tidskr.*, 1916, **10**, 183.
4. Abe, K., *Sci. Rep. Tohoku Univ. Ser. IV*, 1972, **36** (3), 179.

SOME NEW STONE AGE SITES IN THE UPPER MAHANADI VALLEY, MADHYA PRADESH

THE Upper Mahanadi Valley has not been explored much but the lower reaches and its tributaries have been studied by Mohapatra[1] who found a number of Palaeolithic and a few Mesolithic sites in the valley. Later, Tripathi[2] also discovered a number of sites of Palaeolithic and Mesolithic cultures from the tributaries of the lower reaches of Mahanadi Valley.

A large number of stone age sites were discovered by the author in the Raipur District of Madhya Pradesh, during the course of his explorations in that region from January to March, 1977. The new sites discovered are: Manchewa, Paterapali, Mahasamund, Bemcha, Kudar Nadi, Jalki Dongri, Jogideepa, Borid Barbaspur, Sukda, Bhandora, Putpura, Pairagura, Balamdi Nadi, Turibhatha, Thakurdaiya, Parsada, Nandnia, Borsi, Bagar, Goalindih,

The tools found in the present explorations of the Upper Mahanadi Valley belong to the Mesolithic culture. The material utilized in their manufacture is predominantly chert, though chalcedony and quartz have also been used. The tools include blades, points, lunates, triangles scrapers, borers, and cores. While blades, lunates and points have been found in profusion at all the sites, triangles are very rare and trapezes are altogether absent. This fact indicates that the present sites of Raipur District belong to early phase of Mesolithic culture.

Most of the sites are located on the slopes of the hills or on rocky surfaces (Fig. 1). Some, however, are found on the banks of the streams in the alluvial deposits away from the hills. All the sites discovered in the present exploration are in fact factory sites because finished tools are found in less number as compared to the manufacturing waste (cores, flakes, chips, etc.).

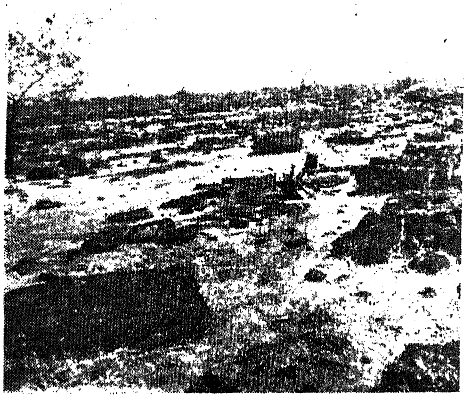

FIG. 1. Hill slope on the right bank of Mahanadi near Bhandora.

The present studies show that large population was inhabiting the hilly slopes in Raipur District during the Mesolithic period. The ecology of the entire area seems to have favoured the hunting-gathering economy of the people.

The Mahanadi Valley therefore provides a rich potential area for detailed investigations.

The author is grateful to Professor R. V. Joshi for his guidance.

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1. Mohapatra, G. C., *Stone Age Cultures of Orissa*, Deccan College, Pune, 1961.
2. Tripathi, K. C., *Lithic Industries of South-Western Orissa.*, 1972, (unpublished Ph.D. Thesis, Utkal University).

FIELD RECOVERY OF *CHELONUS BLACKBURNI* CAMERON (HYMENOPTERA: BRACONIDAE) FROM THE COTTON SPOTTED BOLLWORM *EARIAS VITTELLA* (FABRICIUS)

AMONGST the spotted bollworms, *Earias vittella* (Fabricius) (=*fabia* stoll) is a serious pest of both cotton and bhendi [*Abelmoschus esculentus* (Linnaeus) Moench] in the Karnataka State. Since a species of *Chelonus* has been reported to parasitise the cotton bollworms *E. vittella* and *Earias insulana* Boisduval in India[2], preliminary tests were conducted to find out the efficacy of an egg-larval parasite, *Chelonus blackburni* Cameron in parasitizing *E. vittella* under field condition. A nucleus culture of this species (originally procured from the U.S.A.) was obtained from the Commonwealth Institute of Biological Control, Indian Station, Bangalore and multiplied in the laboratory at the Department of Entomology, College of Agriculture, Dharwar. In the preliminary experiments conducted under laboratory conditions the parasite was observed to readily accept the eggs of *E. vittella*. The adults reared from this host were found quite normal. Further, its efficacy under field conditions was also tested by releasing the freshly emerged adult parasites in small areas of cotton and bhendi which were affected by the spotted bollworm. Parasite releases were made in batches and a total of 4784 adults were released from 27-9-1976 to 24-2-1977. After the lapse of 10 days of each release, young larvae of *E. vittella* were collected at random from released plots and were reared individually till they pupated. Out of 350 larvae of spotted bollworm collected from the field, 4 adults of *C. blackburni* emerged in the laboratory, and parasitisation of *E. vittella* in cotton and bhendi was found to be 1·2% and 1·0% respectively

C. blackburni is an egg-larval parasite of the cotton pink bollworm *Pectinophora gossypiella* (Saunders) in Hawaii[5] and has been successfully introduced against *P. gossypiella* in the U.S.A.[1,3] and in the West Indies[4]. Thompson[6] lists this species as a parasite of *E. insulana* in Egypt which seems to be erroneous. Except this there is no report of *C. blackburni* paratizing the spotted bollworms. It is interesting to ote from the present studies that the parasite has ccepted *E. vittella* as its host under laboratory and eld conditions and recovery of the parasite from he field, though small in numbers, is recorded. Further studies on the parasite are in progress.

The authors are grateful to Dr. T. Sankaran, Entomologist-in-charge, Commonwealth Institute of Bio ogical Control, Indian Station, Bangalore, for supplyng the nucleus culture of *C. blackburni* and Dr. S. V. atil, Director of Instruction (Agri.), College of Agriulture, Dharwar, for kind encouragement.

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1. Bryan, D. E., Fye, R. E., Jackson, C. G. and Patana, R., *Cotton Insects Biol. Control, Lab. ARS., USDA, Tucson*, 1973, p. 8.
2. Husain, M. A. and Mathur, V. B., *Rept. Proc. Fifth Ent. Meeting Pusa*, 1923, p. 34.
3. Noble, L. W. and Hunt, W. T., *J. Econ. Ent.*, 1937, **30** (6), 842.
4. Squire, F. H., The Pink Bollworm (*Platyedra gossypiella*, Saunders), Trinidad, 1939, p. 11.
5. Swezey, O. H., *Proc. Hawaiian Entom. Soc., Honolulu*, 3 (2), 99.
6. Thompson, W. R., *A Catalogue of the Parasites and Predators of Insect Pests*, 1949, section 1, Pt. 6, p. 21.

ON THE MORPHOLOGY OF Y AND OTHER CHROMOSOMES IN THE INDIAN FLYING FOX, *PTEROPUS GIGANTEUS GIGANTEUS*

THE Indian bat commonly called flying fox, *Pteropus giganteus giganteus*, Brunnich, a member of family Pteropidae of order Chiroptera, has already been cytologically investigated[1,2]. Though the diploid number was reported earlier by Manna *et al.*[1] its karyotype was constructed only later[2]. Present study represents further investigation of somatic chromosomes of this species to highlight the morphological differences between autosomes and the sex chromosomes, specially the Y.

The chromosome preparation was done from bone marrow tissues following Colchicine-hypotonic-squash technique[3] with iron-alum haematoxylin staining. The chromosome number was determined from 185 metaphase nuclei, obtained from both male and female specimens. The diploid number was confirmed to be 38. Fig. 1 (A and B) represents karyograms of male and female chromosome complements showing serial alignments of 18 pairs of autosomes numbered in decreasing order of size and the sex chromosomes at the end; among these 18 pairs of autosomes arranged according to the position of the centromere, 10 pairs of metacentrics (nos. 1, 2, 3, 4, 6, 7, 10, 12, 14, 15), 4 pairs of submetacentrics (nos. 5, 8, 13, 17) and 4 pairs of subtelocentrics (nos. 9, 11, 16, 18) are observed. The X chromosome is subtelocentric, but the Y chromosome is a minute metacentric (Fig. 1A, at the arrow).

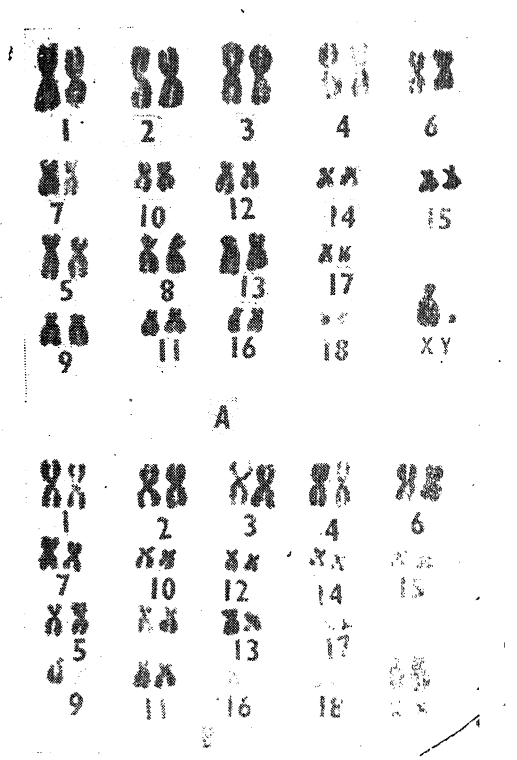

FIG. 1. Karyograms of *P. g. giganteus*. A represents male and B, a female complement. Arrow indicates position of centromere in Y chromosome, × 1,200.

The karyotype analysis of Ray Chaudhuri et al.² in the same species reported 11 pairs of metacentrics, 4 pairs of submetacentrics and 3 pairs of subtelocentrics with subtelocentric X and acrocentric Y. In the present study the author has found that the last pair of the autosomes is subtelocentric contrary to the submetacentric pair reported by Ray Chaudhuri et al.² The structure of the sex chromosomes, specially the Y, is different in appearance; Y is minute dot-like acrocentric in their figure and not very clear. Present study shows distinct gap locating middle position of centromere and proving it to be metacentric. X-chromosome is subtelocentric in both the studies.

The author is thankful to Dr. G. K. Manna, Head, Department of Zoology, Kalyani University, for providing necessary laboratory facilities for this work.

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1. Manna, G. K. and Talukdar, Madhuri, *Mam. Chrom. News Letter*, 1965, **17**, **77**.
2. Ray Chaudhuri, S. P., Pathak, S. and Sharma, T., *The Nucleus*, Seminar on Chromosomes, 1968, p. 96.
3. Ford, C. E. and Hamerton, J. L., *Stain Technol.*, **1956**, **31**, **247**.

ICRO/UNESCO COURSE ON 'ANIMAL VIRUS AND TISSUE CULTURE'

Training course on recent techniques in Cell Research, on Animal Virus and Tissue Culture will be held from January 2–20, 1978, at the Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore.

Participants will carry out fundamental experiments in modern quantitative methods. Lectures, demonstrations, seminars and group discussions will be held with the co-operation of a number of leading specialists.

The number of participants is limited to sixteen. Only post-graduate students in the fields of physics, chemistry and biology (including biochemistry and microbiology), will be accepted. Their living expenses for the duration of the course will be offered by the Indian agencies. A limited number of fellowships, which will cover travel expenses, will be offered by ICRO/UNESCO. Accepted participants will be notified by November 20, 1977.

For details please contact Prof. T. Ramakrishnan, Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore 560012. Deadline for applications. October 31, 1977.

SHORT SCIENTIFIC NOTES

Infrared Studies on K-Vitamins

K-vitamins are important bio-molecules, responsible for blood-coagulation. Hans[1] studied the synthetic homologues of K_1 and K_2 vitamins with a view to characterising them. In this laboratory 2-methyl-1, 4-naphthoquinone, (K_3 vitamin) was spectroscopically studied[2].

Further studies of the various K-vitamins were undertaken for comparison of their spectral properties. The infrared spectra of K_1, K_3, K_4 and K_5 vitamins (E Merck, Germany) in the range 200-4000 cm^{-1} were taken with Perkin-Elmer 621 grating spectrophotometer equipped with NaCl optics. In order to achieve better resolutions, they were scanned at various optimum speeds, the accuracy being about ± 3 cm^{-1}. The bands were analysed on the assumption of the point-group symmetry Cs. The important frequencies belong to CH bending, CH_3 stretching, $C-C$ stretching and $C=O$ stretching modes of vibrations. On the basis of their similarities, it is found that a quinonoid character prevails in the various K-vitamins.

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1. Noll, Hans, *J. Biol. Chem.*, 1958, **232**, 919 and *Ibid.*, 1960, **235**, 2207.
2. Singh, S. Nath, and Singh, R. S., *Indian J. Pure Appl. Phys.*, 1967, **5**, 394 and *Spechim. Acta*, 1968, **24A**, 159.

A Giant Caterpillar that Attacks Apple

Paralebeda flagifera (Walker) (Lasiocampidae: Lepidoptera) which was earlier recorded as a pest of citrus from the hilly region of West Bengal (Mukherjee and Nath, 1971)[1], was noticed for the first time on apple at Mashobra during 1976.

The caterpillar is stout, big (10 cm) and mottled bluish grey with a pair of long tuft of hairs on first thoracic segment which are projected forward. It feeds on the leaves and sometimes chews soft bark of the stem, during April-May. It produces hissing voice when disturbed. Pupation occurs in the first week of June in a big loosely spun tent-shaped cocoon. The adult emerges after 32 days. Moth is 12 cm (wings expanded), brown with irregular broad dark brown lobe like band in the middle and a black spot at the anal margin of fore wings. Female could lay 158-190 smooth, oval and white eggs in clusters, without mating. In the present collection, out of seven caterpillars only one male was obtained which did not mate in captivity. The unfertilized eggs laid by females remained unhatched.

The author is thankful to Dr. G. S. Dogra, Professor and Head, Department of Zoology-Entomology, College of Agriculture, Solan, for encouragements and to Dr. Swaraj Ghai, Sr. Systematic Entomologist, Division of Entomology, I.A.R.I., New Delhi, for identifying the insect.

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RAMESH CHANDER.

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1. Mukherjee, D. and Nath, D. K., *Paralebeda flagifera* (Walker) (Lasiocampidae: Lepidoptera) a new record of citrus pest in West Bengal. *Indian J. Ent.*, 1971, **33** (1), 103.

An Improved Method for Preserving the Marine Algae

The traditional method of preparation of herbarium sheets of marine algae involves sticking the algal material directly on herbarium sheet under pressure. In course of time, such a material gets separated from the sheets and later on becomes useless for observation; moreover the original colour also fades. Sandwitching the alga between two polythene sheets facilitates direct microscopic examination as well as the retention of their original colour for a longer period. This could conveniently be sent by post with very little packing.

The following procedure helps one to get a durable herbarium specimen: Freshly collected algal material, cut to size, is cleaned under running water to remove dirt. A piece of muslin cloth is soaked in water and spread on a glass plate (30 × 45 cm). It is then dipped in water in an enamel tray and the alga is spread on the cloth. The glass plate with the cloth and alga is carefully taken out of the tray and the water is slowly drained off without disturbing the arrangement of the specimen. It is then covered by another piece of cloth. The alga, sandwitched between the cloth pieces, is placed between two blotting sheets and dried by conventional method.

The dried material is separated from the cloth and placed between two polythene sheets covered with plain paper and aluminium sheets. The specimen thus prepared is placed in an electric plant-press kept at 70° C for half an hour. This would seal the alga between the two polythene sheets while the paper ensures that the polythene sheets do not stick to metal plates. The sealed algal material is now ready for observation. Material prepared in this way has been preserved for three years in an excellent condition.

The author is thankful to the Director, Institute of Science, for facilities and Dr. V. D. Tilak and Shri S. S. Kelkar for valuable help.

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Bombay 400 032, *March* 4, 1977.
C. S. LATTOO.

REVIEWS AND ANNOUNCEMENTS

Soil Fertility—Theory and Practice. Ed. J. S. Kanwar. (Indian Council of Agricultural Research, New Delhi), 1976. Pp. 533. Price Rs. 28.

The book is a compilation of 14 chapters written by 15 authors who are specialists in different branches of Soil Science. It deals with several aspects of soil as a medium for plant culture and the soil-plant relationships and the various means of maintaining the relationships at optimum levels. The effect of environmental factors on uptake of nutrient by plants is dealt with in another chapter. Individual nutrients, both major and minor and the organic matter in soil and their importance in plant production as also their uptake replenishment and management are fully covered. There is adequate coverage of the chemical fertilizers. There is also a chapter on 'Land Degradation and Pollution', the importance of which is increasingly realized in this country in recent years.

The book is an exhaustive treatise and is a valuable addition to our knowledge in Soil Science. The illustrations are very apt and well designed. The data given in the innumerable tables add value to the publication. Written by fifteen top specialists in Soil Science, it is rather difficult to build them together into one volume of uniform reading and Dr. J. S. Kanwar has carried out this in an efficient manner. Dealing with various aspects of Soil Fertility and the theory and practice of it, one cannot ignore the microorganisms involved in the maintenance of fertility levels and their role in off-setting pollution problems and several other interactions in soil-plant relationships. One chapter on the basic principles involved in soil-plant microbe inter-relationships and the role of microorganisms in mobilization and immobilization of plant nutrients under the influence of various ecological and agronomic factors would have further added to the value of the book.

G. RANGASWAMI.

Nonlinear Analysis—Theory, Methods and Applications. An International Multidisciplinary Journal, Vol. I, No. I. (Pergamon Press Ltd., Headington Hill Hall, Oxford, OX3-OBW), 1976. Pp. 90. Annual Subscription: Institutions $ 80.00; Individuals $ 30.00.

The very title and the editorial contained in the first volume number is enough to stress the need and usefulness of this journal. On going through the contents of this issue the reviewer no doubt feels that the editor has kept his promise made in the editorial. One looks forward to further issues.

C. UBEROI.

WINTER SCHOOL ON CURRENT TRENDS ON BIOMOLECULAR STRUCTURE UNIVERSITY OF MADRAS

A Winter School on "Current Trends in Biomolecular Structure" is to be held from January 9 to 14, 1978, immediately following the International Symposium on "Biomolecular Structure, Conformation, Function and Evolution" as a part of the Silver Jubilee Celebrations of the Physics Department.

A Series of lectures on the Current state of our knowledge on the Biomolecular Structure and Conformation will be delivered for a week by visiting experts participating in the International Symposium. Lecurtes will deal with Biomolecular Structures, their folding patterns, etc. and techniques such as X-ray diffraction, NMR, IR and Laser Raman Spectroscopy, will also be covered.

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A COMPUTER METHOD FOR PREDICTING THE SEQUENCE OF tRNA FROM ITS ENZYMATIC DIGESTION PRODUCTS BASED ON ITS SECONDARY STRUCTURE

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THE procedure followed for the determination of the sequence of RNA involves digestion of the purified sample with endonucleases of known specificity followed by separation of the fragments and their analysis for individual sequence. The RNA is then partially digested with less enzyme under controlled conditions, to get larger fragments which are again separated and analysed to get sufficient overlaps to deduce the unique sequence. The procedure is laborious as several partial digestions have to be done to arrive at the sequence[1,2]. It is well known that for a given primary sequence of RNA various secondary structures can be predicted[3]. So far no successful method for constructing the primary sequence from the oligonucleotides has been devised, although attempts have been made to deduce the sequence from the oligomers[4]. The possible sequences even for a small chain of 50 nucleotides will run into very large numbers. However, the number of possibilities will be drastically reduced if secondary structure restrictions are imposed. The best choice of applying the computer method would, therefore, be to transfer RNA because of its well established cloverleaf structure[5,6]. We have devised a computer method to simulate all isotomers from the oligonucleotides which are formed by digestion of the RNA with RNase T$_1$ and pancreatic RNase. The application of this method to the determination of the primary sequence of tRNA is illustrated with *E. coli* tRNA$_2^{gln}$ as an example.

PRINCIPLE OF THE COMPUTER METHOD

The principle used is basically a "tree search" method, the flow-chart of which is shown in Fig. 1. The method may be compared to the procedure in finding all the possible routes to the top of a highly branched tree. To reach the top one starts from the stem and goes up the various branches. If one branch starting from a particular node does not lead to the top another branch is tried and if none of the branches from the node leads to the top a branch in a node below is taken; the process is repeated until the top is reached. Essentially the same procedure is followed in the computer for building the nucleotide chain from the fragments. There is a starting fragment similar to the stem from which the chain branches out. After the addition of each fragment the nucleotide chain branches out further into sub-branches. In some cases it will not be possible to build the chain along certain branches, as the chain would stop before all the fragments are used up; certain order of addition as indicated below has to be followed at each stage. The computer programme is to search for the path to be followed, check whether all the fragments are used and print the sequence when the chain is complete. All the possible sequences will be printed out by the computer. The flow-chart (Fig. 1) gives a brief account of the computer operation. Details of the programme will be published elsewhere.

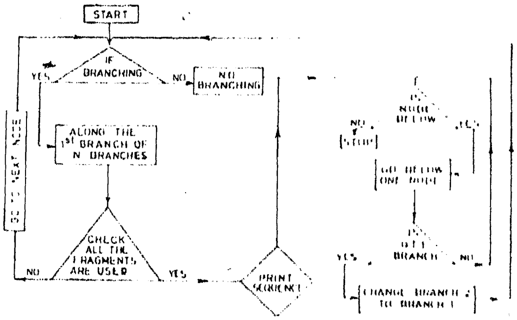

FIG. 1. The flow-chart of the computer programme. Details are given in the text.

If RNA is treated with RNase T$_1$ it will be degraded into fragments which have G$_p$ at the 3′ end. Free G$_p$ will be liberated from stretches of guanylic acid residues in the chain. Similarly upon treatment with RNase A fragments ending in U$_p$ and C$_p$ as well as free U$_p$ and C$_p$ will be produced. G$_p$, U$_p$ and C$_p$ are omitted from the fragments for building the nucleotide chain as these are already present in other fragments. RNase A products will contain all the G$_p$ stretches while those of RNase T$_1$ will contain all the U$_p$ and C$_p$ stretches. In some cases it is possible to get longer sequences by the combination of a RNase T$_1$ fragment and a RNase A fragment from the overlaps[5]. For example, upon digestion of tRNA$_2^{gln}$ (Ref. 7) with RNase T$_1$, a fragment DAAG and with RNase A a fragment G″GD will be formed (D, dihydrouridylic acid residue and G″ methyl-guanylic acid residue, see the structure of, the tRNA, ref. : 8). The two oligonucleotides can be combined to G″GDAAG, the nucleotide D being the overlap. Other overlapping sequences can be combined in a similar manner. Thus the fragments shown in Table I are the various sequences that can be obtained from tRNA$_2^{gln}$ experimentally.

TABLE I

Forward fragments

Fragments of tRNA$_2^{gln}$ read 5′ to 3′.

1.1 pU$\underline{G}$ (2)	3.1 CC$\underline{AAG}$ (4)
2.1 G$\underline{C}$ (3)	3.2 CACC$\underline{G}$ (2)
2.2 G$\underline{U}$ (5)	3.3 CAUUCC$\underline{G}$ (2)
2.3 GG$\underline{C}$ (3)	3.4 CGGD$\underline{AAG}$ (4)
2.4 GGGG$\underline{U}$ (5)	3.5 CCA$_{OH*}$
2.5 GAUUCC$\underline{G}$ (2)	4.1 AAG$\underline{C}$ (3)
2.6 GGAUUCU$\underline{G}$ (2)	4.2 AAGG$\underline{C}$ (3)
2.7 GAGGUψC$\underline{G}$ (2)	5.1 UAUC$\underline{G}$ (2)
2.8 GAAUCCUC$\underline{G}$ (2)	5.2 UACCCCAG$\underline{C}$ (3)

As many overlaps as possible were obtained from the enzymatic fragments from the position of the minor nucleotides[5]. Minor nucleotides, except ψ and D, are represented by the parent nucleotides. The oligomers are arranged In groups. The first number indicates the group to which it belongs, the second its position in the group and the number on the right, in brackets, shows the group of the fragment which follows. The nucleotide(s) underlined shows the overlap with the next fragment. Molar ratio of the fragments is one in all cases.

These fragments are fed to the computer. Construction of the sequence tree is done in both directions starting from either end in a systematic way. It is obvious that in the present case pUG is the starting fragment from the 5′ end. The fragment to be added next to pUG depends on the nucleotide produced from its 3′ end upon treatment of this RNase T$_1$ fragment with the complementary endonuclease, RNase A. Since G will be the nucleotide that will be produced, a fragment starting with G has to be added to pUG, the nucleotide G being the overlap. If GGC from group 2 (see Table I) is added the sequence becomes pUGGC. The oligonucleotide GGC is a RNase A product and on treatment of this with RNase T$_1$ will produce C. Hence a fragment starting with C has to be added to pUGGC. Any of the oligonucleotides in group 3 may be added. If CCAAG is added the overall sequence becomes pUGGCCAAG. It is easily seen that the fragment to be added next is any one of the two fragments in group 4 (see Table I). Thus the sequence tree is built in the forward direction. The nucleotide chain is built in the backward direction from 3′ end on the same principle. In the latter case mirror images of the sequences of the fragments are used to build the sequence. In other words, sequences are read from 3′ to 5′. For example CCA is taken as ACC, GGC as CGG and CCAAG as GAACC. The overlaps between two adjacent fragments is decided in a manner similar to that Indlcated above. Table II shows the reverse fragments with the overlaps..

TABLE II

Reverse fragments

Fragments of tRNA$_2^{gln}$ read 3′ to 5′.

1.1 AC$\underline{C}$ (2)	3.4 GCUA$\underline{U}$ (4)
2.1 C$\underline{G}$ (3)	3.5 GCCA$\underline{C}$ (2)
2.2 CG$\underline{G}$ (3)	3.6 GCCUUA$\underline{C}$ (2)
2.3 CGACCCCA$\underline{U}$ (4)	3.7 GCψUGGA$\underline{G}$ (3)
2.4 C$\underline{GAA}$ (5)	4.1 UGGG$\underline{G}$ (3)
2.5 CG$\underline{GAA}$ (5)	4.2 U$\underline{G}$ (3)
3.1 GCUCCUAA$\underline{G}$ (3)	5.1 GAAC$\underline{C}$ (2)
3.2 GUCUUAG$\underline{G}$ (3)	5.2 GAADGG$\underline{C}$ (2)
3.3 GCCUUA$\underline{G}$ (3)	

Fragments given in Table I are read in the reverse order, grouped and presented. The last fragment GUp is not shown.

The method was programmed for IBM 360/44 in Fortran IV language. The cloverleaf model of tRNA given in the *Handbook of Nucleic Acid Sequences* by Barrell and Clark[8] was used as the secondary structure foe deriving the primary sequence.

RESULTS AND DISCUSSION

The construction of the cloverleaf was carried out part by part with the restrictions imposed. The amino acid stem was constructed first by building the sequence from the 5′ end as well as from the 3′ end and selecting those structures which formed 7 consecutive hydrogen bonds (see Ref. 8). Fragments containing dihydrouridine (D) and pseudouridine (ψ) were omitted for building the stem. tRNAs generally do not contain these nucleotides in the amino acid stem. Hence fragments containing these nucleotides were omitted. It was programmed to simulate all sequences up to a chain length of 8 nucleotides from

5′ end or the nearest number when the last fragment is added. The number of structures thus obtained was 39. In all tRNAs the 8th nucleotide from the 5′ end is U or a modified U (see Ref. 8). Out of the 39 structures only 5 had U at the 8th position. These are shown below :

1. $\text{pUGAAUCC}\underline{\text{U}}\text{CG}$
2. $\text{pUGGAUUC}\underline{\text{U}}\text{G}$
3. $\text{pUGCACCG}\underline{\text{U}}\text{AUCG}$
4. $\text{pUGCACCG}\underline{\text{U}}\text{ACCCCAG}$
5. $\text{pUGGGGUA}\underline{\text{U}}\text{CG}$

The nucleotide chain was also built from 3′ end to generate all sequences up to the 11th nucleotide or the nearest number with the reverse fragments (Table II). Eleventh nucleotide from 3′ end is the last one involved in base pairing on the amino acid stem. A total of 82 structures were obtained. Each of the 5 forward structures shown above was tested to find out whether it would pair with any of the 82 backward structures. Surprisingly only the 5th structure, pUGGGGUAUCG, gave duplex structures containing 7 or more hydrogen bonded pairs with two of the 82 reverse sequences. These are shown below :

A. ACCGACCCCAUGGGGU
I I I I I I I I I I
pUGGGGUAUCG

B. ACCGACCCCAUG
I I I I I I I I
pUGGGGUAU

may be noted that in structure A the oligo-leotide GGGGU is used both in the forward the reverse sequences and hence it was ruled it. Therefore the only structure possible was the second one, B. It is to be mentioned in this connection that *E. coli* formyl methionine tRNA[9] contains only 6 hydrogen bonded pairs in the amino acid stem. Yeast alanine tRNA[5] *E. coli* leucine tRNA_1[10] and wheat germ phenylalanine tRNA[11] ntain one mismatch each in the stem. These re exceptions and were not considered in the present study in selecting the stem from all the structures. However this kind of mismatches also may be taken into account in selecting the stem. GU pair formation was allowed in the present case.

Dihydrouridine and TψCG arms

Our next attempt was to build on to the stem (structure B above) the dihydrouridine arm in the forward direction and TψCG arm in the reverse direction. There are a number of regularities regarding the positions of various nucleotides on tht dihydrouridine arm. It has a hydrogen bonded structure with 3 or 4 pairs and always it is the 10th nucleotide which forms the first hydrogen bonded pair. In the loop region a sequence AG or AA occurs followed by D in a somewhat fixed position (see Ref. 8). Besides the loop contains a GG sequence between 17 and 21 positions. The number of nucleotides in the loop is variable between 7 and 12, the maximum number required to build the chain up to the end of the arm being approximately 27. Therefore, it was programmed to simulate structures up to 27 nucleotides from 5′ end or the nearest number with the last fragment on the chain. Out of the 426 sequences printed out, only two structures satisfied all the restrictions on the dihydrouridine arm. The structures are :

(*a*) $\text{pUGGGGUAUC}\underline{\text{GCC}}\text{AAGCGGDAA}\underline{\text{GGC}}\text{ACCG}$

(*b*) $\text{pUGGGGUAUC}\underline{\text{GCC}}\text{AAGCGGDAA}\underline{\text{GGC}}\text{AUUCCG}$

The nucleotides underlined form base pairs. It may be noted that the difference between the two structures is only in the last fragment ; CACCG in (*a*) is replaced by CAUUCCG in (*b*) (see Table I). Detection of these structures among the 426 structures was not at all difficult as large number of structures which did not satisfy any one of the restrictions on the dihydrouridine arm could be eliminated by just looking at the sequences.

Restrictions on the TψCG arm are even more stringent. The number of paired and non-paired nucleotides in this arm is fixed and the total number up to the last hydrogen bonded pair is 28 from 3′ end (see Ref. 8). The nucleotide, ψ, always appears on 22nd position in the loop region. Besides the 16th nucleotide is C which base pairs with G on the 24th position. The computer program in the construction of the TψCG arm was to simulate structures up to the 28th nucleotide from 3′ end with the reverse sequences, omitting the fragment containing dihydrouridine and those already used in forming the stem. A total of 278 structures were generated. Only 4 structures satisfied the restrictions on the TψCG arm. They are :

(i) $\text{ACCGACCCCAU}\underline{\text{GCUCC}}\text{UAAGC}\psi\text{T}\underline{\text{GGAGU}}\text{CUUAGG}$

(ii) $\text{ACCGACCCCAU}\underline{\text{GCUCC}}\text{UAAGC}\psi\text{T}\underline{\text{GGAGC}}\text{CUUAG}$

(iii) $\text{ACCGACCCCAU}\underline{\text{GCUCC}}\text{UAAGC}\psi\text{T}\underline{\text{GGAGC}}\text{CAC}$

(iv) $\text{ACCGACCCCAU}\underline{\text{GCUCC}}\text{UAAGC}\psi\text{T}\underline{\text{GGAGC}}\text{CUUAC}$.

The nucleotides which are underlined are involved in hydrogen bond formation. These structures differ from each other only in the last fragment.

Completion of cloverleaf

Since two structures (*a* and *b*) in the forward direction and four structures (i to iv) in reverse direction were obtained, a minimum of 8 possibilities for the entire sequence appeared possible. Each of the combination was considered to find whether it would form the anticodon arm when combined with the unused fragments in each case. Combinations (*a*) (iii) and (*b*) (iv) were not possible as the same fragment had been used in the forward as well as in the reverse sequences. It may be noted that (*a*) contains CACCG while (iii) contains GCCAC as the last fragments. The latter, GCCAC, is nothing but CACCG in the forward direction.

For each of the other combinations (*a*) (i), (*a*) (ii), (*a*) (iv), (*b*) (i), *b* (ii) and (*b*) (iii) there were 3 unused fragments. With the forward chain, the reverse chain and 3 unused fragments two complete sequences could be obtained with each of the 6 combinations. Thus a total of 12 sequences could be obtained at the final stage. The anticodon arm has a number of regularities common to all tRNAs. It has 5 hydrogen bonded pairs and 7 unpaired nucleotides. Besides there is one unpaired nucleotide between the dihydrouridine arm and the anticodon arm. Only one sequence from combination (*a*) (iv) satisfied all the common features of the anticodon arm and it had in the loop region a triplet which agreed with the codon for glutamine. The sequence thus obtained is shown below.

10 20 30
pUGGGGUAUCGCCAAGCGGDAAGGCACCGGAUUCUGAU

40 50 60 70
UCCGGCAUUCCGAGGTψCGAAUCCUCGUACCCCAGCCA.

This structure agrees with that in reference 7, the modified nucleotides being represented by the parent nucleotide. None of the other 11 structures obtained could give a complete cloverleaf with its common features. The method is applicable to any tRNA provided the nucleotide sequences of the individual fragments are correctly determined. It is likely that we may not end up with a unique solution in every case. However as the secondary structure constaints on a tRNA are numerous we strongly feel that the number of possible sequences will be small and the method will greatly reduce the time needed to sequence a tRNA. Once the enzymatic fragments are well characterised the time needed to do the computer work is very short. In the present study selec of the correct sequences at various stages was d manually from the computer output. This operat also may be computerised, if needed.

At present, it appears that the method is suita only for tRNA. However, it is applicable to RNA provided sufficient secondary structure co straints are available. A common secondary stru ture with 4 base paired regions applicable to seve species of 5S RNA has been proposed[12]. Wheth this will be sufficient to work out the sequence of RNA by the computer method remains to be te

ACKNOWLEDGEMENT

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1. Holley, R. W., " Prog. in nucleic acid research *Mol. Biol.*, 1968. **8**, 37.
2. Ohashi, K., Harada, F., Ohashi, Z., Nishimur S., Stewart, T. S., Vogeli, G., McCutchan, and Soll, D., *Nucleic Acid Research*, 1976, 3369.
3. Tinoco, I., Uhlenbeck, O. and Levine, M *Nature*, 1971, **230**, 362.
4. Merril, C. R., Shapiro, M. B., Bradley, D. F Mosimann, J. E. and Vinton, J. E., *Biopoly mers*, 1966, **4**, 723.
5. Holley, R. W., Apgar, J., Everett, G. A., Madison J. T., Marquisee, M., Merrill, S. H., Penswick T. R. and Zamir, A., *Science*, 1965, **147**, 146
6. Pipas, J. M. and McMohan, J. E., *Proc. Natl Acad. Sci., U.S.A.*, 1976, **72**, 2017.
7. Folk, W. R. and Yaniv, M., *Nature*, N.B., 1972, **237**, 165.
8. Barrell, B. G. and Clark, B. F. C.. *Handbook of Nucleic Acid Sequences*, Joynson-Bruvvers, Oxford, 1974, p. 5.
9. Dube, S. K., Marcker, K. A., Clark, B. F. C and Cory, S., *Nature*, 1968, **218**, 232.
10. Dube, S. K., Marcker, K. A. and Yudelevich, A., *FEBS Letters*, 1970, **9**, 168.
11. Dudock, B. S., Katz, G, Taylor, E K. and Holley, R. W., *Proc. Natl. Acad. Sci., U.S.A.*, 1969, **62**, 941.
12. Fox, G. E. and Woese, C. R. *Nature*, 19 **256**, 505.

A QUALITATIVE APPRAISAL OF THE REGIONAL MAGNETIC INVESTIGATIONS OVER THE NORTH CUDDAPAH BASIN

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ABSTRACT

The Cuddapah basin of Andhra Pradesh has been of considerable geophysical interest since the observation of high negative Bouguer gravity anomalies over it, despite zero or positive density contrast of the basin sedimentaries with the surrounding Archaean country. The results of the magnetic investigations conducted over an area of about 20,000 square kilometres over and around Cuddapah basin north of 16° parallel are presented and discussed qualitatively. The basin is brought out as a separate and distinct unit in contrast with the surroundings in the magnetic anomaly map. A probable mid-basin upliftment is suggested. A huge deep seated body with a possible causative connection with the mineralisation at the eastern margin of the Cuddapah basin is proposed.

INTRODUCTION

THE Cuddapah basin, which is typically sedimentary, occupies a crescent shaped tract of about 13,500 square miles in area in the east-central part of Peninsular India. It is an epicontinental basin formed after a considerable lapse of time since the deposition, upliftment and metamorphism of Dharwars. It is believed that the basin originally extended beyond its present boundaries, to the west and possibly also to the north (Krishnan[6]).

The special geophysical interest in the basin is the observation of high negative Bouguer gravity anomalies over the basin sedimentaries of nearly equal or slightly greater densities compared to the surrounding country. Glennie[2-4], had attributed these gravity anomalies to a down-warping of the crust. Glennie's explanations, however, did produce neither clinching evidence for the crustal down-warp nor for an overthrust in the east.

The geophysics department of Andhra University has already conducted large-scale geophysical investigations, with a close network of gravity and magnetic observations, over Godavari Valley, Gondwana basin, Cuddapah basin and parts of Eastern ghats. In this paper, results of the magnetic investigations conducted over the North Cuddapah basin and the adjoining area are presented and discussed qualitatively.

GEOLOGY OF THE AREA

Major part of the North Cuddapah basin consists of the older Cuddapah series, while the Kurnools are exposed in the southern and south-western sides of the area. Srisailam quartzites of Kistna series are found in the western part of the basin extending from the western boundary right upto the centre of the basin and as a rim enclosing it round the northern tip, and the eastern part, upto Nekarikallu. Outliers of Srisailam Quartzites are well recognised on the eastern as well as the western sides of the basin. Achempet dome is one of such outliers in the east. Limestones are extensively developed in the area. Cumbum Shales, are developed in the north and also in the south of the basin, along the eastern margin. All along the eastern margin, they are highly folded, contorted and overturned.

The other formations occurring in the area are a patch of Kurnools, consisting of Paniam Quartzites, Kundair Limestones, Banganapalle Quartzites in the central area. Unclassified quartzites are exposed at different places in the basin. In the south western side, Vempalle Limestones and Gulcheru Quartzites, Pulivendla/Nagari Quartzites, Tadpatri/Pullampet Shales are exposed.

MAGNETIC SURVEYS

The present investigations cover an area of about 20,000 square km., between 16° and 17° N latitudes and 78° and 81° 15′ E longitudes. Nearly 900 magnetic stations were set up at 3 to 6 km intervals in the area. Every attempt has been made to see that the station distribution is even, as far as possible, in the area. An Askania Torsion Magnetometer of type GfZ was used for making the magnetic observations.

Reduction of the Magnetic Data.—The magnetic anomalies presented here are computed with reference to the primary base station at Vijayawada. The base is established after an exhaustive study of the area and its vicinity and after continuous magnetic observations on a number of days at the beginning of the surveys. Number of auxiliary bases have also been set up during the course of the survey for operational convenience and for the computation of the correction for the diurnal variation of the earth's vertical magnetic intensity. These auxiliary bases are duly tied magnetically with the primary base by the standard method (Nettleton[8]).

For the computation of diurnal correction, a typical diurnal variation pattern is obtained on a day at each of the auxiliary bases by making observations at quarter-hourly intervals. The variations obtained were then compared with the Magnetic Observatory data of the National Geophysical Research Institute, Hyderabad, on the corresponding days and suitable adjustments in the magnitude of the variations for the auxiliary bases, the type of variation remaining same, were made. During the remaining days, observations at the beginning and end of the day's work were only made at the base and diurnal variations curves for these days are obtained as outlined and used for the computation of the diurnal correction.

The normal correction is evaluated by bringing forward the gradients of vertical field intensity in the north-south and east-west directions for the whole area under survey from the maps and tables published by the Carnegie Institute of Washington (Vestine *et al.*[9]). For the area under survey, the average values of gradients in the north–south and east–west directions adopted respectively are 16 and 0·6 gammas per km. The magnetic anomalies thus computed are contoured and presented in Fig. 1.

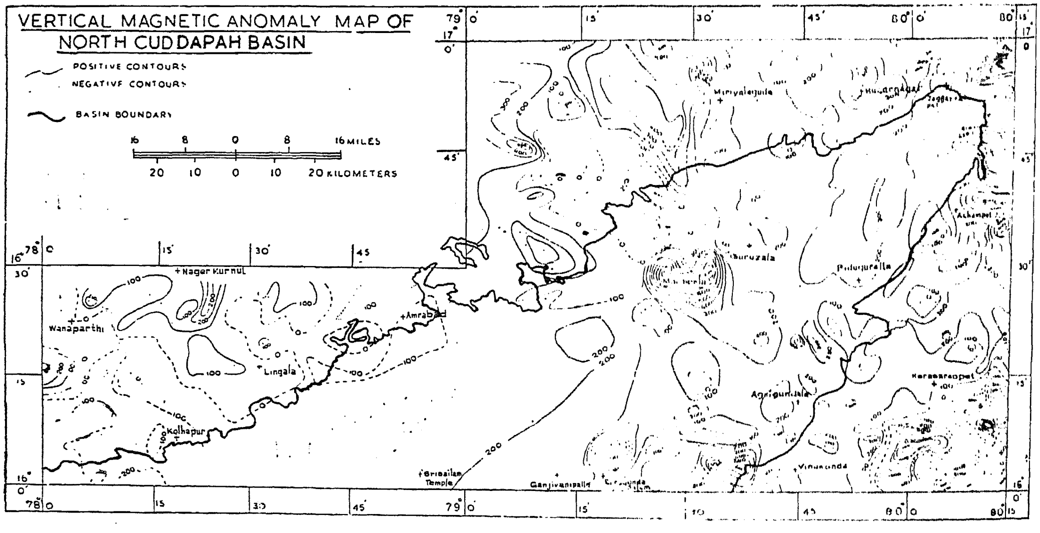

FIG. 1. Vertical Magnetic Anomaly map of North Cuddapah Basin.

DISCUSSION OF THE ANOMALIES

The regional magnetic picture shows that the area covered by sedimentaries is quite well represented with very few magnetic anomalies. In contrast with the area outside, the basin is magnetically flat and magnetic gradient could be observed across the boundary on all sides of the basin. An elongated + 200 gamma anomaly contour in the Palnad area roughly corresponds with the margins of the basin. To the south and south-west of the area also a number of + 200 gamma contours could be seen. It, thus, appears that a positive anomaly of 200 gammas seems to be a characteristic anomaly associated with the basin. On the other hand, at some places especially north of Piduguralla, and coming down further to the south, quite a few anomalies of the order of + 300 gammas are observed indicating a broad magnetic high in the middle of the basin. These anomalies are supposed to be associated with the basement configuration, in the form of a possible mid-basin upliftment.

In the western part of the basin, the distribution of the magnetic anomalies seems to be somewhat different from that on the eastern part. Between Amrabad and Wanaparti, the magnetic anomalies are positive on the crystallines west of the basin and negative within the basin, while a zero anomaly contour seems to correspond roughly with the boundary of the basin. The magnetic contours in this area are widely spaced indicating gentle gradients. It is also observed that the magnetic anomalies on the crystallines west of the basin in this area do not exhibit, in general, the intense magnetic fluctuations observed on the crystallines east of the eastern margin of the basin.

While discussing the numerous outliers of the Cuddapahs, Foote[1], and others have suggested possible extension of the basin to the east of the present eastern margins. The distribution of the magnetic anomalies in the eastern part of the basin and on the crystallines east of the eastern margin seems to suggest that the magnetically flat area of the basin extends to east, beyond the eastern margin before intense magnetic fluctuations are encountered. The magnetically flat zone corresponds roughly to the area enclosed by the eastern margin of the basin and a line passing through Vinukonda, Narasaraopet and Achampet. Considerably less pronounced feature is noticed west of the western margin of the basin.

Intense positive and negative anomalies of the order of + 1400 gammas and — 1200 gammas in the area around Macherla have been observed. These anomalies could not be considered as due to some local cause because of the consistency of their range and their distribution over a large area around Macherla. The disposition of the observations is also fairly satisfactory there, to rule out the possibility of any local cause. Hence, this anomaly feature has to be explained not as due to any superficial cause but as related to the structures near or underlying the basement. This area has been studied in some detail and a possible relationship between the mineralisation occurring near the eastern margin of the basin and the high magnetic anomalies, observed here, has been worked out (Narasimhaswamy[7]).

Foote[1] had observed a singular series of elliptical anticlinal domes, six in number along the eastern boundary of the basin extending from Vinukonda nearly upto the Krishna river. It is difficult to say, with the present data, whether or not these domes have found any representation in the isoanomaly map. But the Vinukonda dome composed of post-syntectonic granites seems to have some relationship with the high positive anomalies of the order of + 1300 gammas south of it, whereas the Ipuru dome of the same composition as that of the Vinukonda dome has not found any significant mark in contour map. The apparent effect of the Nekarikallu dome composed of slates and phyllites, and the Biravallipaya outlier still to the north, both of them occurring near Piduguralla, seems to be + 300 gamma anomaly, west of the actual domes. The Achampet faulted dome, a true outlier of the sedimentaries nto the gneissic country, is marked by a zero nomaly contour in the south-west and + 600 gamma ontour in the north-east. The inability to get exact correlation of the anomalies with the domes, and their wide displacement in relation to the domes might perhaps be due to the effect of numerous basic dykes in the surrounding country rock adjacent to the basin.

In the southern part of the area, in the middle of the basin near Erragondapalem and in the east, beyond Vinukonda, roughly along and above the 16° parallel, across the basin, magnetic disturbance could be observed with negative centre near Erragondapalem followed by positive anomalies and then intense negative anomalies in the east near Chilakaluripet. Heron (1947) has suggested the existence of two basins of subsidence, the larger one including the main area of Cuddapahs and the Kurnools, and the smaller one, the Palnad group. He advocated that in the Palnad basin, subsidence may have commenced later, not long before the deposition of Cuddapahs had finished in the main basin, and continued with far greater regularity and in deeper water as is shown by the immense accumulation (?) of the uniform limestones and shales interrupted only by the diamondiferous grits. His ideas, according to many succeeding investigators, are questionable and impracticable. However, a probable correlation between the above-mentioned feature in the observed anomaly map and the suggested boundary separating the two basins is apparent.

There is a marked contrast in the magnetic anomalies of the areas east and west of the basin. The area east of the basin right from Jaggayyapet in the north up to Chilakaluripet in the south is characterised by intense magnetic fluctuations. The anomalies north-east of Jaggayyapet are of local nature caused by the float iron ore deposits. Anomalies south of Jaggayyapet and up to Chilakaluripet indicate partly the intense tectonic disturbance in the form of folding, crushing, overturning, and faulting along the eastern boundary of the basin. The igneous activity in this area resulting in numerous basic dykes such as dolerites, may account for these anomalies to a greater extent.

Comparatively, the area west of the basin is not as disturbed magnetically as the eastern area. But in the area, west of Mirialguda and north of Macherla, occupied by crystallines, the magnetic anomalies exhibit fairly high gradients and magnitudes.

CONCLUSIONS

Distribution of observations was not satisfactory at some places, especially around Srisailam, because of dense forest and highly irregular terrain resulting in very scarce habitation and lack of any development of roads, etc. Despite these limitations in the data, the magnetic anomaly map has been able, to a large extent, to represent the various features observed by the geologists, and to bring out a picture of the basement relief. The basin has been shown as a separate and distinct unit from the rest of the area indicating the extended boundaries. The major eastern boundary fault propounded by King[5] has found its representation on the anomaly map. Results of further analysis of the data and the quantitative studies made, have also broadly conformed to the general qualitative interpretation obtained herein.

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Thanks are due to Prof. E. A. R. Deutsche for his valuable comments on the work done and to Dr. M. Gangadhara Rao for his help in the conduct of the field work. Financial assistance of the Council of Scientific and Industrial Research, India, is gratefully acknowledged.

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1. Foote, R. B., "On the Geological structure of the Eastern Coast from Latitude 15° Northward to Masulipatnam," *Mem. Geol. Surv. Ind.*, 1880, 16.
2. Glennie, E. A., *Gravity Anomalies and Structure of the Earth's Crust*, Surv. Ind. Prof. Paper No. 27, 1932.
3. —, "Crustal warping," *Mon. Not. Roy. Astr. Soc. Geophys. Suppl.*, 1933, 3.
4. —, "The Cuddapah Basin in India and crustal warpings," *Ibid.*, 1951, 6.
5. King, W., "The Cuddapah and Karnool formations in the Madras Presidency," *Mem. Geol. Surv. Ind.*, 1872, 8.
6. Krishnan, M. S., *Geology of India and Burma*, Higginbothams, Pvt. Ltd., Madras, 1960.
7. Narasimhaswamy, G., "Regional magnetic investigations in North Cuddapah Basin," *Ph.D. Thesis*, 1970 (Unpublished).
8. Nettleton, L. L., *Geophysical Prospecting for Oil*, McGraw-Hill, Book, Co., Inc., New York, 1940.
9. Vestine, E. H., Laporte, L., Cooper, C., Isabelle, L. and Hendrix, W. C., *Description of the Earth's Main Magnetic Field and its Secular Change*, 1905–1945, Carnegie Institution of Washington Publication, 1959, p. 578.

SOME PHYSICOCHEMICAL PROPERTIES OF N-BENZENESULPHONYL L(—) HISTIDINE AND RELATED LIGANDS

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ABSTRACT

The syntheses of N-benzenesulphonyl L(—) histidine ($R^h H_2$), α-benzenesulphonamido β-2-benzimidazolyl L(—) *n* propionic acid ($R^p H_2$) have been reported and the uv and ir spectral data recorded. Preparation of α-benzenesulphonamido γ-2-benzimidazolyl L(+) *n*-butyric acid ($R^b H_2$) has also been described. Ir data show that these compounds exist as zwitterions.

COMPLEX compounds of various metal ions with histidine and related ligands exhibit very interesting properties[1-6]. However no attempt has yet been made to study the complexing behaviour of benzenesulphonyl derivative of histidine. The present paper describes the synthesis of N-benzenesulphonyl L(—) histidine ($R^h H_2$) and α-benzenesulphonamino β-2-benzimidazolyl L(—) *n*-propionic acid ($R^p H_2$). Their electronic spectra in ethanol and ir spectra in KBr matrices are also recorded. The corresponding data of α-benzenesulphonamide γ-2-benzimidazolyl L(+) *n*-butyric acid ($R^b H_2$) are included for comparison[7]. All these compounds exist as zwitterions and are found to form complexes with Cu(II), Ni(II) and Co(II) and may function as monoprotic or biprotic tridentate ligands. Details of complexation is under investigation.

EXPERIMENTAL

N-Benzenesulphonyl L(—) histidine ($R^h H_2$).—L(—) Histidine was allowed to react with benzenesulphonyl chloride in the presence of warm alkali. The reaction product was treated with norit, filtered and acidified with warm acetic acid (pH ~ 3), when shining crystals of $R^h H_2$ separated. It was recrystallized from hot water, m.p. 267° d, slightly soluble in water, ethanol; soluble in DMF, DMSO, etc. (Found: Eq. Wt. 296·5. $C_{12}H_{13}O_4N_3S$ requires Eq. Wt. 295·0). UV spectrum λ_{max}^{EtOH} 285 nm (log ε 3·71), 274 nm (3·78), 267 nm (3·81) and 215 nm (4·01).

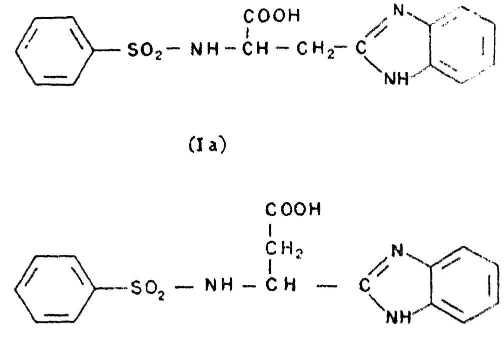
α-Benzenesulphonamido β-2-benzimidazolyl L(—) n-propionic acid ($R^p H_2$)—N-Benzenesulphonyl L(—) aspartic acid (0·11 mole) and *o*-phenylenediamine (0·2 mole) in 60 ml 4N hydrochloric acid were gently boiled under reflux for 4 h. After cooling the pH of the solution was adjusted to ~ 9 first with NaOH and finally with NH_4OH. The compound separated was collected by filtration and found to be 2-2′ (benzenesulphonamido) ethylene dibenzimidazole[9]. By adjusting the pH of the filtrate to about 3 with acetic acid, a white crystalline compound is separated. The compound was dissolved in dilute ammonia and reprecipitated by adding acetic acid and finally recrystallised from warm aqueous DMSO. White shining crystals were obtained, m.p. 226° d, slightly soluble in water and ethanol but highly soluble in DMF, DMSO, etc. (Found : C, 52·30 ; H, 4·80 ; N, 11·60 ; $C_{16}H_{15}O_4N_3S$, H_2O requires C, 52·89 ; H, 4·68 ; N, 11·57%). UV spectrum λ_{max}^{EtOH} 280 nm (log ϵ 3·70), 275 nm (3·81), 243 nm (3·76) and 223 nm (3·93). If to the filtrate after separation of 2–2′ (benzenesulphonamido) ethylene dibenzimidazole, 40 ml of ethanol were added and then acidified by acetic acid, shining white crystals were obtained, m.p. 198° d. It is soluble in water, ethanol, DMF, DMSO, etc. (Found : C, 58·20 ; H, 5·20 ; N, 15·30. $C_{22}H_{23}O_4N_5S$ requires C, 58·28 ; H, 5·08 ; N, 15·45%). UV spectrum λ_{max}^{EtOH} 277 nm (log ϵ 4·88), 271 nm (4·87) and 226–227 nm (5·08).

α-Benzenesulphonamido γ-2-benzimidazolyl L(+) n-butyric acid (R^{b} H_2).—This compound is a monobenzimidazole derivative of N-benzenesulphonyl L(+) glutamic acid[8] and was prepared following the method reported in our previous communication[7]. An equimolecular mixture of *o*-phenylenediamine and monopotassium salt of N-benzenesulphonyl L(+) glutamic acid[10] (0·04 mole each) in 45 ml 4N HCl was boiled under reflux for 4 h. The pH of the cooled solution was adjusted to ~ 9 first with NaOH and finally with NH_3 and filtered. On acidification of the filtrate (pH ~ 4) by acetic acid, a white crystalline compound separated. It was recrystallised from warm aqueous DMSO, m.p. 236° d, slightly soluble in water, ethanol, highly soluble in DMF, DMSO, etc. (Found : Eq. Wt. 358·0 ; C, 57·03 ; H, 4·91 ; N, 11·73, $C_{17}H_{17}O_4N_3S$ requires Eq. Wt., 359·0 ; C, 56·82 ; H, 4·73 ; N, 11·69%). UV spectrum λ_{max}^{EtOH} 281 nm (log ϵ 3·72), 275 nm (3·77) and 235–236 nm (3·72).

UV spectra were measured with a PYE UNICAM spectrophotometer. IR spectra were obtained with a BECKMAN IR-20 spectrophotometer in KBr disks. The pk values were determined using BECKMAN pH meter at constant ionic strength (μ = 0·5) at 31 ± 1°. Melting points recorded were uncorrected.

DISCUSSION

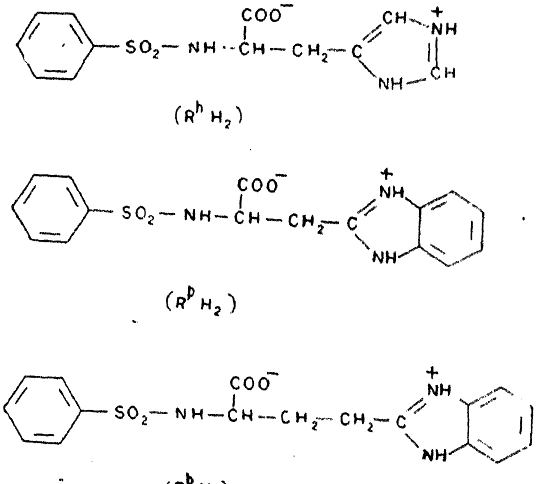
Analytical data indicate that the compound $C_{16}H_{15}O_4N_3S$ is a monobenzimidazole derivative of N-benzenesulphonyl L(—) aspartic acid which may be represented by any one of the two alternative ways (I *a*) or (I *b*) (Fig. 1). Comparing the pk_{COOH} (2·48) of $C_{16}H_{15}O_4N_3S$ with that for the starting material N-benzene sulphonyl L(—) aspartic acid (pk_3 2·84, pk_2 4·41)[11] and pk benzimidazolium ion (5·60) with that for some known compounds[7], it is obvious that this compound is actually (I *a*). The compound $C_{22}H_{23}O_4N_5S$ is a salt of $R''H_2$ and *o*-phenylenediamine.



(I a)

(I b)

FIG. 1



(R^h H_2)

(R^b H_2)

(R^b H_2)

FIG. 2

Electronic spectra of the compounds show a number of peaks in the uv region, which are closely comparable to those found in substituted benzimidazoles[12]. IR spectral data show that N-benzenesulphonyl L(+) glutamic acid and N-benzenesulphonyl L(—) aspartic acid exhibit bands in the region ~1700–1724 cm^{-1} due to COOH. But in these three compounds ($R^{h}H_2$, $R^{p}H_2$ and $R^{l}H_2$) no band in this region is observed. In addition bands in the regions 1570–1613 cm^{-1} and 1360–1369 cm^{-1} due to COO^- antisymmetrical and symmetrical stretches respectively are observed[12-13]. These indicate the zwitterion structures for the compounds which may be represented as in Fig. 2.

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1. Perrin, D. D. and Sharma, V. S., *J. Chem. Soc.*, 1967, A, p. 724.
2. Csovari, S. and Lichner, D., *Med. Radi* 1969, 14, 28.
3. Rogozina, E. M., Popov, D. K. and Ponikro T. M., *Zh, Obshch. Khim.*, 1968, 38, 19
4. Chawla, I. D. and Andrews, A. C., *J. Inor Nucl. Chem.*, 1970, 32, 91.
5. Morris, P. J. and Martin, R. B., *J. Am Chem. Soc.*, 1970, 92, 1543.
6. Michaitiois, M. S. and Martin, R. B., *Ibid* 1969, 91, 4683.
7. Ghosh, N. N. and Nandi, M. M., *J. India Chem. Soc.*, 1976, 53, 274.
8. Hedin, S. G., *Ber. dt. chem. Ges.*, 189 23, 3197.
9. Ghosh, N. N. and Nandi, M. M., *J. India Chem. Soc.*, 1976, 53, 331.
10. — and Dasgupta, M., *Z. Anorg. Allg. Chem* 1970, 375, 315.
11. — and Nandi, M. M., *J. Indian Chem. Soc.* 1976, 53, 317.
12. Williams, D. H. L. and Flemming, I. *Spectroscopic Methods in Organic Chemistry* McGraw-Hill, New York, 1966.
13. Bellamy, L. J., *The Infrared Spectra of Complex Molecules*, Methuen, London, 1954.

ISOLATION AND CHARACTERISATION OF ANTHOCYANIN PIGMENT FROM PHOSPHORUS-DEFICIENT MAIZE PLANTS

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ABSTRACT

Phosphorus-deficiency in maize (*Zea mays* var. Ganga–5) resulted in the accumulation of anthocyanin pigment in leaves. The accumulating pigment was extracted in methanol-HCl (99:1) and a part of it was hydrolyzed to separate the aglycone (anthocyanidin) and the sugar moieties. The purified anthocyanin pigment and its aglycone were subjected to chromatographic and spectrophotometric analyses and the pigment was identified as cyanidin-3-glycoside, a monoside. Sugar moiety was identified as rhamnose. On the basis of these studies, the accumulating pigment was characterized as cyanidin-3-rhamnoside.

INTRODUCTION

IDENTIFICATION of anthocyanin pigment in maize has so far been accomplished only in the aleurone layer of seeds[1] and the anthers of Pr strains[2]. But no information is available on the identity of the pigment accumulating in the leaves of phosphorus-deficient maize. It was, therefore, decided to undertake the identification of the accumulating pigment.

EXPERIMENTAL

Phosphorus-deficiency was developed in maize (*Zea mays* var. Ganga-5) plants, grown by sand culture technique[3,4], by supplying them 0·3 ppm of phosphorus in the nutrient medium, instead of 62 ppm supplied for normal growth. After 40 days of growth, the pigment was extracted from the leaves in methanol–HCl (99 : 1 v/v) and purified chromatographically[5]. A part of the purified pigment was acid hydrolyzed[6] and the aglycone (anthocyanidin) was collected in 2–3 ml of amyl alcohol and purified[5]. The aqueous layer of the hydrolysate contains large amount of hydrochloric acid besides the sugar fraction. Before analysis, the acid was removed from the sugar sample[7].

The *Rf* values of the anthocyanin pigment and its aglycone were studied by descending chromatography on Whatman No. 1 paper. The solvents

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used for anthocyanin pigment were: BAW (Butanol-acetic acid-water; 4 : 1 : 5 v/v, upper layer), BuHCl (Butanol-2-N HCl; 1 : 1 v/v, upper layer) and 1% HCl. The solvents used for the aglycone were: Forestal (Conc. HCl-acetic acid-water; 3 : 30 : 10 v/v), Formic (Conc. HCl-formic acid-water; 2 : 5 : 3 v/v) and BAW. The absorbance of the anthocyanin pigment and its aglycone were recorded in u.v. and visible parts of the spectrum. Finally the bathochromic spectral shift of the pigment was studied in the presence of aluminium ions at pH 2–4. All the experiments dealing with characterisation of anthocyanin pigment, were done in the dark. The sugar fraction of the pigment was also identified chromatographically[8] and by studying the spectrum of the aniline-oxalate complex of the sugar[9].

RESULTS AND DISCUSSION

The results of the present work are summarized in Table I. The *Rf* values of the extracted pigment in different solvents and the λ_{max} values in u.v. and visible range of the spectrum tallied, very closely with those of 3-monosidic glycosides of cyanidin. The pigment also underwent a bathochromic spectral shift, the value being very close to that obtained with standard cyanidin glycosides. This indicated that the pigment had free *o*-dihydroxylic groupings, which further strengthened its identity as cyanidin based pigment. Unlike 5-glycoside and 3, 5-diglycoside, this pigment does not fluoresce in u.v. light and gives dull colour. It was thus taken to be an additional proof for the pigment being a 3-glycoside, a monoside.

The *Rf* values of the aglycone fraction in different solvents and the λ_{max} values in u.v. and visible range of the spectrum also tallied exactly with those of authentic cyanidin. Finally, the *Rf* value of the sugar fraction, the colour of its aniline oxalate complex and the spectral absorbance of the complex were exactly similar to those observed with standard rhamnose.

From the results of the present work, it can be seen that the pigment extracted from the leaves of phosphorus-deficient maize plants has the following structural features (Fig. 1): (*i*) The aglycone part of the pigment is cyanidin. (*ii*) The pigment is a 3-glycoside, a monoside. (*iii*) The sugar present in the pigment is rhamnose. It is, therefore, evident that the extracted pigment

TABLE I

Properties of the anthocyanin pigment extracted from maize leaves

Anthocyanin pigment	*Rf* (× 100) in			λ_{max} in		
	BAW	BuHCl	1% HCl	U.V.	Visible	AlCl$_3$ shift (nm)
Anthocyanin from *Zea mays*	31	42	10	284	533	46
Cyanidin based pigment	31	44	9	284	533	45

Anthocyanidin pigment	*Rf* (× 100) in			λ_{max} in	
	Forestal	Formic	BAW	U.V.	Visible
Anthocyanidin from *Zea mays*	49	21	64	277	535
Authentic anthocyanidin	49	21	68	277	535

Sugar	*Rf* (× 100) in Benzene-Butanol-Pyridine-Water (3 : 10 : 5 : 4 v/v)	λ_{max} in the range 330–550 nm
Sugar from the extracted pigment	83	370
Standard rhamnose	83	370

is cyanidin-3-rhamnoside. Thus a novel pigment is being reported from the maize plants.

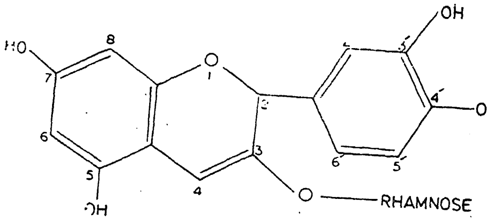

FIG. 1. Cyanidin-3-rhamnoside.

ACKNOWLEDGEMENTS

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1. Harborne, J. B. and Gavazzi, G., *Phytochem.*, 1969, 8, 999.
2. Styles, E. D. and Ceska, O., *Ibid.*, 1972, 11, 3019.
3. Hewitt, E. J., *Sand and Water Culture Methods used in Plant Propagation*, Commonwealth Agricultural Bureax, Farnham Royal. Buck. England, 1966, p. 56.
4. Arnon, D. I. and Hoagland, D. R., *Soil Sci.*, 1940, 50, 463.
5. Harborne, J. B., *Biochem. J.*, 1957, 70, 22.
6. —, *Comparative Biochemistry of Flavonoids*, Academic Press, London, 1967, p. 1.
7. Saini, A. S., *J. Chromat.*, 1966, 24, 484.
8. Hais, I. M. and Macek, K., *Paper Chromatography*, Publishing House of Czechoslovak Academy of Sciences, Prague, 1963, p. 289.
9. Horborne, J. B., *Phytochemical Methods—A Guide to Modern Techniques of Plant Analysis*. Chapman and Hall, Ltd., London, 1973, p. 59.

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LETTERS TO THE EDITOR

A PRELIMINARY NOTE ON CARBONATITE IN WAH SUNG VALLEY OF JAINTIA HILLS DISTRICT, MEGHALAYA

Introduction

A PYROCHLORE-BEARING carbonatite was recently discovered in the Wah Sung Valley of Jaintia Hills District, Meghalaya, during exploration by the Atomic Minerals Division of Department of Atomic Energy and detailed investigations are currently under way for evaluation of its rare-metal potential. This carbonatite complex has a large cover of weathered soil rich in pyrochlore and is apt to be a source for production of niobium similar to some of the carbonatites of Africa.

Location

The carbonatite occurs in an area of bowl-shaped setting, ranging in elevation from 880 m to 1500 m m.s.l. (Lat.: 25° 32′ 15″–25° 36′ 45″; Long.: 92° 06′ 30″–92° 08′ 30″; toposheet No. 83 C/NW) in the Wah Sung Valley of Meghalaya. It is about 25 km east of Shillong and is approachable from 45 km post on the Shillong–Jowai road (NH–44).

eologic Setting

The rocks of the area are pre-cambrian gneisses nd meta-sediments of Shillong group, represented by uartzite, phyllite and quartz-sericite schist. A small utlier of Cherra sandstone of tertiary age lies in the uth-eastern parts of the area. The pre-cambrians ccupy higher elevations whereas the central depreson of the valley is mainly underlain by an ultramafic neous complex composed of dunite, mica-peridotite, roxenite, nepheline, syenite, ijolite and gabbro. otite pegmatites are occasionally noted. The area largely under a thick mantle of soil and alluvium d is densely wooded.

rbonatite

Although major portion of the carbonatite is covered brown coloured soil rich in pyrochlore, some hill pes after excavation showed that the carbonatite curs as small irregular injections in the ultramafic mplex and as dykes close to its periphery. They 60–110 m long, 10–30 m wide and display sharp ntacts with the host-rock. They show varying trends, dominant ones being in E–W, NW–SE and NNE–W directions, although parallelism among a few ces is also conspicuous. In general the carbonatite pears to be a ring complex surrounding the central ramafics.

hey are usually medium to coarse grained leucotic rocks composed essentially of calcite with interspersed fluorapatite, serpentinised olivine and accessory fluorite, pyrochlore, perovskite, magnetite and melanite. Chalcopyrite, pyrrhotite, pyrite, ilmenite, strontionite and phlogopite are some of the other minerals noticed in the carbonatite.

These carbonatites are broadly of two categories—the more homogeneous but less radioactive type, weathering to pale earthy colour and the more heterogeneous and radioactive variety, weathering often to chocolate brown material. Some of the carbonatite bodies exhibit structures of flow banding.

Rare Metals

Preliminary analysis of rock and soil samples from the carbonatite complex has indicated the presence of Nb, Ta, U, Ce and phosphate content in significant concentrations.

High radioactivity, mainly due to pyrochlore, is found to be usually associated with a 15–50 cm wide marginal zone of carbonatite along carbonatite-host-rock boundary. Pyrochlore occurs as cubes and octahedras in shades of yellowish brown to reddish brown colour. Partial chemical analysis* of a sample of pyrochlore, separated from soil from Layem nulla, is given below :

Per cent					
U_3O_8	ThO_2	Nb_2O_5	Ta_2O_5	TiO_2	CaO
2·2	8·5	48·6	2·5	0·3	14·0

The dark chocolate-brown coloured soils, capping the carbonatite, are also highly radioactive and consist mainly of magnetite, fluorapatite (50–60% by weight) and clayey matter along with pyrochlore, perovskite, zircon, garnet, pyriboles and altered iron-oxide minerals. Fluorapatite is identified by X-ray diffraction. Preliminary analyses of a few rock and soil samples from the carbonatite are tabulated below. These indicate high contents of Nb, Sr, Ce and Y but vere low Rb in all the samples. Further, some of thy samples show higher uranium content with little or negligible thorium.

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* Nb and Ti are separated from Ta by solvent extraction using T.B.P. After purification of the isolated fractions and estimation of Ti by absorption spectrophotometry, the values of Nb, Ta and Ti are obtained. The coefficient of variance is 5% for Nb_2O_5 and 2% for Ta_2O_5.

*	LE–854 soil	GB–35 soil	GB–37 soil Tereya-tlaim	GB–38 soil Shang-pet	LE–581 and LE–582 Mica peridotite with carbonatite Umtecham	
X-ray fluorescence (ppm except where indicated in %)						
Nb	2·44%	3·43%	411	187	318	474
Ta	393	476	300†	300†	300†	300†
Y	83	84	236	118	33	24
Sr	1755	1581	1612	1265	3368	1799
Ba	415	538	440	519	707	956
Ce	012	1440	2629	1372	207	175
Rb	15	10†	10†	22	28	53
Zn	431	..	..	..	253	183

*	LE–584 soil Layem	GB–35 soil Layem	GB–37 soil Tereya-tlaim	GB–38 soil Shang-pet	LE–581 and LE–582 Mica peridotite with carbonatite Umtecham	
Spectrographic (ppm)						
Ni	36	..	..	..	8	26
Cr	14	..	..	..	14	17
CO	42	..	..	..	11	16
V	194	..	..	..	49	21
Cu	85	..	..	..	12	58
Mn	1·25%	..	..	..	3216	2469
Ti	1288	..	..	..	1188	832
Chemical (present)						
P_2O_5	..	27·12	25·10	22·20		
SO_4^{2-}	..	0·1†	0·1†	0·1†		
Rare earth oxides (Total ignited)		1·0†	1·0†	1·0†		
U_3O_8	..	0·06	0·008	0·004		
TiO_2	..	..	0·8	0·07		
Radiometric (per cent)						
eU_3O_8	0·10	..	..	..	0·016	0·022
U_3O_8	0·065	..	..	..	0·015	0·018
ThO_2	0·12	..	..	..	0·009	0·013

.. not determined. * analysed by AMD Labs., Hyderabad. † less than.

Discussion

The Wah Sung valley carbonatite appears to be a "closed type" as per classification of Smirnov[1] and may hence be expected to extend considerably downwards widening at depth. Preliminary geological fieldwork has established that the ultra-mafic rocks occurring as a plug are surrounded by the ring complex of the carbonatite bordered by the alkalic rocks which could be products of fenitization due to metasomatism of the granite gneisses, the hosts in which the complex is intrusive. Further work is in progress. Mineralogically, it seems to belong to the rare-metal rare-earth category, akin to the Barreiro deposits of Brazil (Smirnov[1]). The discovery of this carbonatite is therefore, not only of geological interest on account of its geographic location in the north-eastern end of the Indian Shield, but also significant as possible large source of pyrochlore and other rare-earth minerals for niobium requirements in the future.

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1. Smirnov, V. I., *Geology of Mineral Deposits*, Mir Publishers, Moscow, 1976, p. 138.

THE EVALUATION OF POLAR AND STERIC EFFECTS ON THE RATES OF METHOXIDE ION CATALYSED CONDENSATION OF SOME *o*-SUBSTITUTED BENZALDEHYDES WITH 3, 5-DIMETHYL-4-NITROISOXAZOLE IN METHANOL MEDIUM

IN continuation of our earlier work[1,2] on the base catalysed condensation of different mono and disubstituted benzaldehydes with 3,5-dimethyl-4-nitroisoxazole, the steric effects on the above reaction were studied by varying the substituents (*viz.*, NO_2, Br, Cl, CH_3 and $O–CH_3$) in the ortho position of benzaldehyde. The reaction was followed spectrophotometrically at $\lambda = 400$ nm at which the 5-styryl derivative (end product of this reaction) obeys Beer's Law. The bimolecular rate constants (k''), polar (P) and steric (S) parameters for all the reactions studied are presented and discussed. Applicability of Taft's equation ($\log k/k_0'' = \sigma^* \rho^* + \delta E_s$) for ortho substituents and quantitative separation of polar and steric effects of these substituents are some of the new features not reported earlier.

All the chemicals used were of AR grade and wherever necessary further purification was done by standard methods. The 3, 5-dimethyl-4-nitroisoxazole was prepared by the literature method[3]. The kinetic features employed are similar to those reported earlier[1].

In the present study, the kinetic results have been interpreted in the light of the mechanism suggested in our earlier work[2]. The reactions were studied in the temperature range of 25° to 40° C. The bimolecular rate constants are listed in Table I. These results indicate that electron withdrawing substituents such as NO_2, Cl, Br groups in the benzaldehyde moiety facilitate the reaction in comparison to ortho methyl benzaldehyde which is taken as a standard, whereas electron donating substituent, such as methoxy group, retard the reaction.

To study the applicability of Taft's equation for ortho substituents, ($\log k''/k_0'' - \delta E_s$) values were plotted against σ^* values at 35° C according to equation:

$$\log k''/k_0'' = \sigma^* \rho^* + \delta E_s. \tag{1}$$

where k'' and k_0'' represent the rate constants for the condensation of any ortho substituted benzaldehyde and *o*-tolualdehyde respectively, σ^* and E_s are the polar and steric substituent constants and ρ^* and δ, the reaction constants for the same two effects respectively. Using the method of solving the simultaneous equations[4], it was found that the kinetic results for all the ortho substitutents studied in the present work could be fitted well into equation (1) with $\rho^* = 0{\cdot}85 \pm 0{\cdot}04$, $\delta = 0{\cdot}14 \pm 0{\cdot}02$ and $\log k_0'' = -0{\cdot}76 \pm 0{\cdot}01$. Although the plot of $\log k''/k_0''$ *vs.* σ^* was found to be linear with a correlation coefficient of $r = 0{\cdot}97$, it is not a satisfactory one. Hence, it was thought worthwhile to plot the values of ($\log k''/k_0'' - \delta E_s$) *vs.* σ^* which gave a good straight line with a correlation coefficient $r = 0{\cdot}98$, showing the presence of both polar and steric effects on this reaction. It was found that the calculated values of $\log k''/k_0''$ according to equation (1) correlated well with the experimental values within the limits of experimental error. This correlation indeed shows in essence the presence of both steric and polar effects in this reaction. The calculated and experimental values of $\log k''/k_0''$ are presented in Table I.

TABLE I

Temperature: 35° *C*

σ^*, E_s, log (k_p''/k_{or}''), $\triangle\triangle G^{\neq}$ and ($P + S$) values

Sl. No.	Substituent	$k'' \times 10^2$ l. mole^{-1} sec^{-1}		σ^*	E_s	log (k_p''/k_{or}'')	$\triangle\triangle G^{\neq}$ (P + S)		log $^0(k''/k'')$ Expl.	log (k''/k_0'') Cald.
		k_p''	k_{or}''				Cal. mole^{-1}	degree^{-1}		
1.	$-OCH_3$	18·3	13·3	−0·22	0·99	0·13	..	..	−0·11	−0·05
2.	$-CH_3$	20·3	17·3	0·00	0·00	0·06	0·00	0·00	0·00	0·00
3.	$-Cl$	70·3	45·3	0·37	0·18	0·20	−597	−482	0·41	0·34
4.	$-Br$	..	43·3	0·38	0·01	..	−563	−460	0·39	0·33
5.	$-NO_2$	100·0	78·7	0·97	−0·75	0·10	−932	−1024	0·66	0·72

Note—k_p'' values are taken from our earlier data[2].

The positive ρ^* value ($\rho^* = 0{\cdot}85$ at 35° C) show that the reaction is facilitated by low electron density at the reaction site. Also the ρ^* value is different and lower than ρ value (0·98) at 35° C obtained in our earlier work[2] for the condensation of meta substituted benzaldehydes, with isoxazole under similar conditions. This indicates that in addition to the polar effects, the rates of the reactions of ortho substituted benzaldehydes are affected by steric factors also. It was observed that the δ (= 0·14) value in the present study is approximately equal to the difference in ρ and ρ^* values (0·98 − 0·85 = 0·13) indicating the steric effect of the ortho substituents. The value of log (k_p''/k_{or}'')* gives an approximate measure of the steric effects of ortho substituents[5]. It is known that if the value of log (k_p''/k_{or}'')* is nearer to σ^* value of the substituent, polar effect is predominant and if it is nearer to E_s value, only steric effect is present. If the value of log (k_p''/k_{or}'') is in between the value of σ^* and E_s, then both polar and steric effects may be assumed to be operative. The latter trend was observed for the reactions studied in the present work. The values of log (k_p''/k_{or}'') are listed in Table I.

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* where k_p'' and k_{or}'' are the rate constants for para and ortho substituted benzaldehyde respectively.

It was also seen that the difference in free energy of activation $\Delta\Delta G^{\neq}$ of substituted benzaldehyde to *o*-tolualdehyde was more or less equal to the sum of individual polar and steric interaction energies (P + S). The polar and steric interaction energies were calculated using the equations[6] $P = -2 \cdot 303\, \sigma^* \rho^*$ RT and $S = -2 \cdot 303\, \delta E_s$ RT, where R and T have the usual significance. The values of $\Delta\Delta G^{\neq}$ and (P + S) are presented in Table I. Even though the values of $\Delta\Delta G^{\neq}$ are small and within the experimental error, the correlation with (P + S) values and the trends are unmistakable. Such trends were also observed by Taft[6] in the methanolysis of 1-methyl esters in methanol medium.

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---

1. (Mrs.) Sushama, K., Sethuram, B. and Navaneeth Rao, T., *Curr. Sci.*, 1974, **43** (7), 209.
2. Jagannadham, V., (Mrs.) Sushama, K., Sethuram, B. and Navaneeth Rao, T. (Communicated).
3. Morgan, G. T. and Burgess, H. B., *J. Chem. Soc.*, 1921, p. 653.
4. Anderson, R. L. and Bancroft, T. A., *Statistical Theory in Research*, McGraw-Hill Book Co., Inc., New York, N.Y., 1952, Chapter 14.
5. Taft, Jr., R. W., Newman, M. S., and Verhoek, F. H., *J. Amer. Chem. Soc.*, 1950, **72**, 4511.
6. Pavelich, W. A., and Taft, Jr., R. W., *Ibid.*, 1957, **79**, 4935.

NIACIN—A POSSIBLE PRECURSOR OF PYRIDOXINE, STUDIED IN *ASPERGILLUS NIDULANS*

A NUMBER of attempts have been made to study the biosynthesis of pyridoxine using mutant strains of *E. coli* requiring pyridoxine for growth and it has been reported that pyruvate, glycerophosphate[1] and phosphoserine[2] may be possible precursors. In our studies on the genetics and biochemistry of seven non-allelic pyridoxineless mutants of *Aspergillus nidulans* (isolated in our laboratory), one mutant strain (y_1 ; *pyro*$_8$) was able to grow on minimal medium supplemented with nicotinic acid. In the present report, more evidence is given for a possible role of niacin in giving its pyridine nucleus for pyridoxine molecule.

The liquid minimal medium was that of Pontecorvo *et al*[3]. The pyridoxine in the mycelia or culture filtrate was quantitatively estimated by microbiological method using *Saccharomyces carlsbergensis*[4]. Culture filtrate had negligible amount of pyridoxine.

The mutant strain y_1 ; *pyro*$_8$ was separately grown in minimal medium, supplemented with optimal level of niacin (800 µg/10 ml) and pyridoxine (0·88 µg/10 ml). In the mycelia, pyridoxine was assayed for a period of 7 days. The results are presented in Fig. 1.

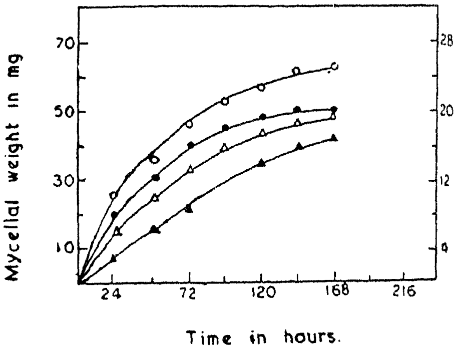

FIG. 1. Mycelial weight and pyridoxine content of y_1 ; *pyro*$_8$ in 10 ml MM supplemented with 800 µg of nicotinic acid.

○——○	Mycelial weight obtained with 0·8 µg of pyridoxine/10 ml of medium.
●——●	Mycelial weight obtained with nicotinic acid (800 µg/10 ml) in the medium.
▲——▲	Pyridoxine content of the mycelium grown in nicotinic acid expressed as µg/gm dry weight of mycelia.
△——△	Pyridoxine content of the mycelium grown in pyridoxine expressed as µg/gm dry weight of mycelia.

In another set of experiments, the mycelia grown in niacin were washed and resuspended in minimal medium alone and minimal medium with niacin or pyridoxine and the results are presented in Fig. 2. From the results presented in Fig. 1, it can be seen that the weight of mycelia as well as the pyridoxine content in mycelia with niacin in the medium is less than in pyridoxine in the medium. However, when the mycelia are transferred to minimal media (Fig. 2), the pyridoxine level decreases progressively, while the pyridoxine concentration increases in the niacin supplemented, reaching the level as with pyridoxine supplemented, suggesting that niacin is converted to pyridoxine in the mutant. There are reports that pyridoxine is formed from glycerol molecules by way of glyceraldehyde-3-phosphate and pyruvate[1]. An alternative pathway of niacin biosynthesis from glyceraldehyde-3-phosphate in addition to the normal pathway from tryptophan and 3-hydroxyanthranilic acid is suggested in *E. coli*[5]. Experiments are under way using other pyridoxineless mutants to find out the effects of 3-carbon compounds, niacin precursors and also to find out the mode of substitution in the pyridine nucleus of niacin to give rise to pyridoxine.

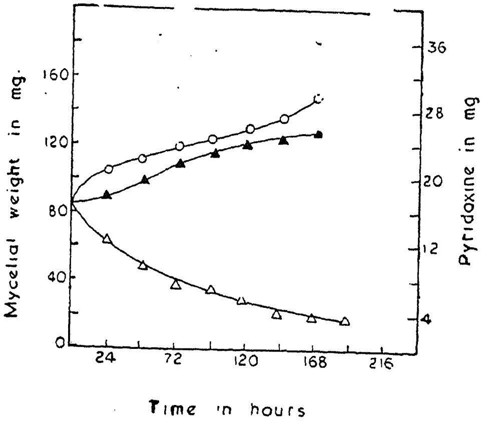

FIG. 2. Mutant y_1 ; *pyro*$_8$ grown in 10 ml MM supplemented with nicotinic acid. Resuspended in MM, MM + nicotinic acid, MM + pyridoxine.

△——△	Pyridoxine content in mycelium during nicotinic acid starvation (grown in nicotinic acid resuspended in MM).
▲——▲	Pyridoxine content in mycelium grown in nicotinic acid and resuspended in nicotinic acid.
○——○	Pyridoxine content of mycelium grown in nicotinic acid resuspended in pyridoxine.

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---

1. Dempsey, W. B., *Biochem. Biophys. Res. Comm.*, 1969, **7**, 89.
2. Hill, R. E. and Spencer, I. D., *Science*, 1970, **169**, 773.
3. Pontecorvo, G., Roper, A. J., Hemmons, L. M., McDonald, K. D. and Bufton, A. W. J., *Adv. Genetics*, 1953, **5**, 141.
4. Morris, J. G., Hughes, D. T. D. and Mudler, *J. Gen. Microbiol.*, 1959, **20**, 566.
5. Ortegal, M. V. and Brown, J. M., *J. Biol. Chem.*, 1960, **235**, 2939.

Ni(II), Cu(II), Pd(II) AND VO(II) CHELATES OF N-HYDROXYETHYL, α-METHYL, 2-HYDROXY-BENZYLIDENEIMINE SCHIFF BASE

N-HYDROXYETHYL, α-methyl, 2-hydroxybenzylideneimine Schiff base (H_2B) functions as a bidentate ligand and forms solid chelates with Ni(II), Cu(II), Pd(II) and VO(II). These chelates have been characterised by elemental analysis, magnetic susceptibility measurements, electronic absorption and I.R. spectra.

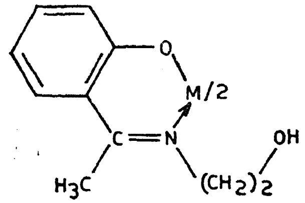
H_2B was prepared by refluxing an equimolecular mixture of *o*-hydroxy acetophenone and ethanolamine in benzene for two hours at 120° C. Water was removed azeotropically with benzene which was in turn removed by distillation when a yellow solid residue was obtained. It was recrystallised (m.p. 134° C) from benzene. It gave satisfactory C, H and N analysis.

The metal-chelates were synthesised by refluxing a mixture of hydrated metal acetates and the ligand in 95% ethanol for nearly two hours and the solid mass formed was filtered and recrystallised from ethanol. On analysis the solid chelates were found to possess 1 : 2 metal-ligand stoichiometry. In addition, the VO(II) chelate possesses one water molecule.

Cu(II) and VO(II) chelates were found to be paramagnetic (μ_{eff} 1·87 and 1·68 B.M. respectively at 303° K) while Ni(II) and Pd(II) chelates diamagnetic.

The absorption spectra of Ni(II) chelates in donor and non-donor solvents consist of two bands around 15900 and 17700 cm^{-1} indicating its square-planar configuration[1]. Similar solutions of the Cu(II) chelate exhibit two broad bands at 16800 and 14500 cm^{-1} which suggest its square-planar geometry[2]. The absorption spectra of the Pd(II) chelate in donor and non-donor solvents consist of three bands at 22500, 26800 and 30700 cm^{-1} indicating its square-planar configuration[3]. The solution spectra of the VO(II) chelate in similar solvents exhibit two bands at 11300 and 19500 cm^{-1} assignable to the transitions[4] $^2B_2 \rightarrow {}^2E_1$ and $^2B_2 \rightarrow {}^2B_1$ respectively in the octahedral environment of VO(II)[5]. Thus H_2B acts as a bidentate ligand in all these compounds.

In the I.R. spectra of H_2B, two bands were observed at 3610 and 1640 cm^{-1} assignable to νOH and νC = N respectively. In the chelates, νC = N was shifted to lower frequency side in a narrow range of 1620–1610 cm^{-1} showing the involvement of the azomethine nitrogen in chelation[6]. The appearance of a new broad band in the range of 650–620 cm^{-1} suggest M–N bonding in the chelates. In the case of VO(II) chelate the loss of a water molecule occurs at relatively high temperature (> 170° C) which indicates that the water molecule is coordinated and not lattice held. Thus the metal chelates may be represented by the structure shown in Fig. 1.



WHERE M = Ni(II) Cu(II) OR Pd(II)
BUT WHEN M = VO(II) ONE MOLECULE OF H_2O IS ALSO COORDINATED TO IT

FIG. 1. Metal chelates of N-hydroxyethyl, α-methyl, 2-hydroxybenzylideneimine schiff base.

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---

1. Cotton, F. A. and Willkinson, G., *Advanced Inorganic Chemistry*, 1966, p. 881.
2. Sacconi, L., *J. Inorg. Nucl. Chem.*, 1961, **19**, 73.
3. Yamada, S., *Coordin. Chem. Rev.*, 1966, **1**, 415.
4. Poddar, S. N., Ghosh, S. and Das, A. K., *J. Indian Chem. Soc.*, 1976, **53**, 788.
5. Selbin, J., *Chem. Rev.*, 1965, **65**, 165.
6. Daasch, L. W. and Hanninen, U. E., *J. Am. Chem. Soc.*, 1950, **72**, 3673.

VINYL POLYMERIZATION INITIATED BY PEROXYDIPHOSPHATE—VANADYL ION REDOX SYSTEM

STUDIES involving peroxydiphosphate for the initiation of vinyl polymerization have been undertaken by us in order to evaluate the use of this compound as a suitable water-soluble initiator. Our preliminary investigation[1] with peroxydiphosphate—Ag^+ redox system revealed some interesting features and it was shown that peroxydiphosphate can function as initiator in the presence of H^+ and suitable reducing agents.

In the present investigation, we report some of the important aspects of another redox system, peroxydiphosphate—VO^{2+}, for the initiation of vinyl polymerization. Edwards *et al.*[2] studied the oxidation of vanadyl ion by peroxydiphosphate and showed that the reaction proceeds *via* complex formation and a free-radical mechanism. It is also evident[3] that V^{5+} alone, in the absence of a reducing agent, cannot initiate the polymerization of a vinyl monomer. Hence the primary radical-producing reaction can be indicated as follows ;

$$VO^{2+} + H_2P_2O_8^{2-} + H_2O \rightleftharpoons \text{Complex} \xrightarrow{k_d} VO_2^{+} + HPO_4^{2-} + HPO_4^{\overline{\cdot}} + 2H^+. \quad (1)$$

This would result in the formation of $HPO_4^{\overline{\cdot}}$ as the only active initiating species. $HPO_4^{\overline{\cdot}}$ by itself can initiate the polymerization of the vinyl monomer or it can react with water producing $\dot{O}H$, a second initiating species[1].

$$HPO_4^{\overline{\cdot}} + M \xrightarrow{k_i} M_1^{\cdot} \quad (2)$$

$$HPO_4^{\overline{\cdot}} + H_2O \xrightarrow{k_{d_2}} H_2PO_4^{-}\dot{O}H \quad (3)$$

$$\dot{O}H + M \xrightarrow{k_{i_2}} M_1^{\cdot} \quad (4)$$

It is not possible to get reactive metal ion intermediate in this system. But in the case of PP–Ag^+ (PP = peroxydiphosphate) redox system, Ag^{2+} formed as the active intermediate would react with water producing $\dot{O}H$ and regenerating Ag^+. In PP–Co^{2+} redox system also, a similar reactive intermediate is formed. In addition to the fact that no reactive metal ion intermediate is formed, V^{5+} formed in the radical-producing step can act as a linear terminator. Termination by the metal ion in its quadrivalent state would also be expected since both oxidative and reductive termination of polymer radicals[5–9] by metal ions are possible. Partial destruction of the initiating species by the reaction of the following type,

$$HPO_4^{\overline{\cdot}} + VO^{2+} + H_2O \rightarrow HPO_4^{2-} + VO_2^{+} + 2H^+ \quad (5)$$

would also be expected.

Experiments were carried out under the conditions, [PP] = $1 \cdot 5$–$3 \cdot 0 \times 10^{-4}$M, [VO^{2+}] = $2 \cdot 5$–25×10^{-3}M,]Acrylonitrile] = $0 \cdot 1519$–$0 \cdot 9112$M and pH = $1 \cdot 95$ (using phosphate buffer) at 25° C under deaerated conditions. Vanadyl sulphate is E. Merck sample. Other experimental details are the same as described previously[1].

Summarising the important observations of the present study, PP–VO^{2+} redox pair initiated the polymerization of acrylonitrile efficiently. A transition in order, with respect to vanadyl ion, $0 \cdot 5$ order at low [VO^{2+}] ($0 \cdot 0025$–$0 \cdot 005$M) and $0 \cdot 1$ to zero order at high [VO^{2+}] ($0 \cdot 005$–$0 \cdot 025$M) was noticed. Over the wide range of [VO^{2+}] ($0 \cdot 0025$–$0 \cdot 025$M), only a first order variation with respect to monomer was observed. A half-order dependence on PP at low [VO^{2+}] ($0 \cdot 005$M) changed into $0 \cdot 9$ order dependence at high [VO^{2+}] ($0 \cdot 025$M). A three-fold increase in hydrogen ion concentration and a two-fold increase in ionic strength did not result in any change in the rate of polymerization. Increase in temperature led to an increase in the rate of polymerization unlike the case of PP–Ag$^+$ redox system.

The above experimental findings indicate that at low concentration of the metal ion, mutual termination takes place exclusively and as the metal ion concentration increases, termination by vanadyl ion or V^{5+} also sets in. As the order tends toward zero with respect to VO^{2+} at high [VO^{2+}], it is probable that termination by vanadyl ion takes place.

$$\text{Polymer radical} + VO^{2+} \rightarrow \text{Polymer} + V(V). \qquad (6)$$

The operation of both mutual and linear terminations would explain the transition in order, with respect to the metal ion.

Since the radical-producing step leads to the formation of the phosphate radical ion only, without any reactive metal ion intermediate and also the initiating species would be destroyed by reaction (5), one would expect PP–VO^{2+} redox system to be less effective than PP–Ag$^+$ redox system. But the reverse is found to be true. This may be due to the ease of oxidation of vanadyl ion relative to Ag$^+$, giving rise to a higher value of k_d and hence the rate of polymerization.

Studies involving various PP-reducing agent redox systems are in progress in developing the efficiency of PP as a water-soluble initiator.

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1. Hariharan, S. S. and Meenakshi, A., *J. Polym. Sci., Polym. Lett.*, 1977, **15**, 1.
2. Anderson, M., Edwards, J. O., Green, A. A. Sr. and Wiswell, M. D. Sr., *Inorg. Chim. Acta*, 1969, **3**:**4**, 655.
3. Saccubai, S., *Ph.D. Thesis*, Madras University, January 1968.
4. Hariharan, S. S. and Meenakshi, A., Unpublished results.
5. Dainton, F. S. and Seaman, P. H., *J. Polym. Sci.* 1959, **39**, 279.
6. Collinson, E., Dainton, F. S., Smith, D. R., Trudel, G. J. and Tazuke, S. (in Part), *Discussions Faraday Soc.*, 1960, **29**, 188.
7. Watanabe, M. and Kiuchi, H., *J. Polym. Sci.*, 1962, **58**, 103.
8. Cavell, E. A. S. and Meeks, A. C., *Polymer*, 1967, **8** (2), 79.
9. Viswanathan, S. and Santappa, M., *J. Polym, Sci., Polym. Lett.*, 1968, **6**, 629.

VAN ECK AND COOK METHOD FOR THE EVALUATION OF THE FORMATION CONSTANT OF METAL CARBONYLS

THE formation constant of some mononuclear and substituted mononuclear carbonyl complexes have been evaluated using a relation derived from Van Eck[1] and Cook equations[2]. The formation constant of four binuclear carbonyl complexes have also been calculated.

Van Panthalcon Van Eck[1] gave an empirical relation between the formation constant of the metal carbonyls and their ionization potential, *viz.*,

$$\log K = 1{\cdot}25\ I - 4{\cdot}2. \qquad (1)$$

D. Cook[2] correlated the stretching frequency of the carbonyl group in metal carbonyls with the ionization potential,

$$\nu C = O = 29{\cdot}20\ I + 1409 \qquad (2)$$

K, $\nu C = O$ and I are the formation constant, stretching frequency of the carbonyl group and ionization potential respectively. The combination of equations (1) and (2) gives the following relation,

$$\log K = 0{\cdot}042\ \nu C{=}O - 64{\cdot}428. \qquad \mathbf{(3)}$$

Equation (3) has been utilized by many workers[3-5] to evaluated the formation constant of various metal carbonyls with their stretching frequency and is found in good agreement with the experimental values. In the present work we have evaluated the formation constant of some mononuclear and substituted mononuclear carbonyl complexes and the data are shown in Tables I and II respectively. Table I also contains the formation constant of four binuclear carbonyl complexes, *viz.*, $Mn_2(CO)_{10}$, $Cr_2(CO)_{10}$, $Re_2(CO)_{10}$ and $W_2(CO)_{10}$. A plot of log K *vs.* $\nu C = O$ gives a straight line in accordance with equation (3). Although the experimental values of the formation constants of these metal carbonyls are not available in literature, the result can be verified in an indirect way. Thus from Table I it is evident that $Ni(CO)_4 > Co(CO)_4^{-} > Fe(CO)_4^{-2}$ are isoelectronic and their formation constants follow the order $Ni(CO)_4 > Co(CO)_4^{-} > Fe(CO)_4^{-2}$. This is due to the fact that a negative charge on the metal atom favours the shift of electrons from the metal *d*-orbital to antibonding * orbital or carbon in CO group, thus decreasing the triple bond character in CO which decreases the bond strength and hence the stretching frequency of the carbonyl group and the formation constant also decreases. The formation constant of Ni > Co > Fe is in agreement with the Irving William[6] series. Similarly a positive charge on the metal atom is reluctant to back-bond formation and hence the formation constant increases in this case. In Table II, of the substituted metal carbonyls of Fe, Mn, Cr and V are isoelectronic and have a general formula $[C_6H_5M(CO)_3{}^n]$ wherein, is the formal

TABLE I

Sl. No.	Metal carbonyls	Stretching frequency $\nu(C=O)$ cm^{-1}	Log K
1.	$Cr(CO)_6$	2100	23·772
2.	$V(CO)_6$	1860	13·692
3.	$Mn(CO)_6^+$	2090	23·352
4.	$Ni(CO)_4^-$	2060	22·092
5.	$CO(CO)_4^-$	1890	14·952
6.	$Fe(CO)_4^{--}$	1790	10·752
7.	$Mn_2(CO)_{10}$	2074	23·108
8.	$Cr_2(CO)_{10}$	1945	17·262
9.	$Re_2(CO)_{10}$	2049	21·630
10.	$W_2(CO)_{10}$	1944	17·220
11.	$Fe(CO)_5$	2034	21·000
12.	$Mn(CO)_5^-$	1898	15·288

TABLE II

Sl. No.	Metal carbonyls	Stretching frequency $\nu(C=O)$ cm^{-1}	Log K
1.	$C_6H_5\ Fe(CO)_3^+$	2120	24·612
2.	$C_6H_5\ Mn(CO)_3$	2035	21·042
3.	$C_6H_5\ Cr(CO)_3^-$	1876	14·364
4.	$C_6H_5\ V(CO)_3^{--}$	1748	8·98
5.	dien $Cr(CO)_3$	1900	15·372
6.	dien $Mn(CO)_3^+$	2020	20·412
7.	dien $Mo(CO)_3$	1883	18·658
8.	dien $W(CO)_3$	1873	14·238

oxidation state having the values + 1, 0, − 1 and −2 respectively. Thus Fe having a positive charge should be most stable whereas vanadium bearing − 2 charge should be least stable and the results obtained are in agreement with this argument, *i.e.*, $-C_6H_5Fe(CO)_3^+ > C_6H_5Mn(CO)_3 > C_6H_5\ Cr(CO)_3^- > C_6H_5\ V(CO)_3^{-2}$. Similarly, dien substituted $Mn(CO)_3$ having a positive charge is most stable. Thus the back-bonding theory proposed for metal carbonyls on the basis of stretching frequency data by F. A. Cotton[7] finds a further support from the formation constant data evaluated.

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1. Van Panthaleon Van Eck, C. L., *Recl. Trav. Chim Pays. Bas Bely*, 1953, 72, 50.
2. Cook, D. J., *Am. Chem. Soc.*, 1958, 80, 49.
3. Singh, P. P. and Pande, I. M., *Indian J. Chem.* 1973, p. 390.
4. — and —, *J. Inorg. Nucl. Chem.*, 1972, 34 1131.
5. Jain, S. C. and Rivest, R., *Ibid.*, 1967, **29**, 2787.
6. Irving, H. and Williams, R. J. P., *Nature*, London. 1948, **162**, 746.
7. Cotton, F. A. and Wing, R. M., *Inorg. Chem.*, 1965, p. 4314.

SUITABILITY OF MYCOBACTERIA AS FOOD FOR FREE-LIVING AMOEBAE

FREE-living aerobic amoebae have been implicated in causing fatal exogenous amoebiasis in man[1] and pulmonary lesions in cattle[2]. Some species of amoebae belonging to *Acanthamoeba-Hartmannella* group have also been isolated from the upper respiratory tract of man suffering from constant headache, cold and nose bleeding[3,4]. Skocil, Cerva and Serbus[5] have reported carrier states for hartmannellid infection in man where the amoebae were isolated from the same subjects on many occasions. No relationship could be observed between the presence of amoebae and the symptoms evidened in the host[6]. The reported occurrence of endoparasitic bacteria in trophozoites of *Acanthamoeba culbertsoni* by Proca-Ciobanu and Jonescu[7] from mouse brain tissues adds new dimensions to the problem of free-living amoebae infecting man and animal. It is likely that free-living amoebae may cause disease in man by themselves and (or) act indirectly by acting as vectors of microorganisms potentially pathogenic to these hosts. A detailed study has been initiated towards understanding the relationship between hartmannellid amoebae and microorganisms known to cause diseases of respiratory tract in man. This communication deals with suitability of mycobacteria as food for *A. castellanii.*

Cultures of *A. castellanii* were maintained axenically in peptine broth (2·0% protease peptone in distilled water, pH 6·7) and subcultured at ten day intervals. The cultures were incubated at 30° C.

The strains of mycobacteria used in this study are: *Mycobacterium tuberculosis* [strains $H_{37}Rv$ (TMC 102), Erdman (TMC 107), $H_{37}Ra$ (TMC 201)], *M. bovis* [strains Vallee (TMC 409), B.C.G., (Phipps) TMC 1029], *M. avium* (Kirshberg TMC 801/J);

Photochromogens—*M. kansasii* (Brownell, TMC 1203), *M. marinum* (ATCC 927); Scotochromogens *M. scrofulaceum* (Gause TMC 1316); Non-photochromogens—*M. terrae* (TMC 1450), *M. triviale* (TMC 1453), *M. gastri* (TMC 1456), *M. xenopi* (TMA 1470); Rapid growers—*M. smegmatis* (TMC 1456), *M. phlei* (TMC 1523), *M. vaccae* (TMC 1526), *M. diernhoferi* (TMC 1540), *M. flavescens* (TMC 1541), *M. chelonei* (TMC 1542), *M. fortuitum* (TMC 1529); mycobacteria encountered in lower warm blooded animals namely: *M. microti* (TMC 1608) and *M. ulcerans* (Tub/71) along with *M. simiae* (TMC 5134) a species *incertae sedis*. Stock cultures of mycobacteria were maintained in Lӧwenstein and Jensen's media. For test purposes the mycobacterial cultures were grown in a dispersed state in modified Proskauer and Beck liquid medium containing 0·05% (vol./vol.) tween 80. Strains of *M. tuberculosis*, *M. bovis* and *M. kansasii* were grown in media enriched with 10% bovine serum. *M. marinum* and *M. ulcerans* were maintained at 33° C while the rest of the strains were incubated at 37° C.

The effect of mycobacteria on *A. castellanii* was tudied in the following manner: 2·0% (wt./vol.) oxoid sagar No. 3 was prepared in distilled water (pH 6·7), sterilized by autoclaving at 15 lb/sq. in. pressure for 15 min., and poured into sterile petridishes. Sterile glass rings (2·5 cm in diameter) were then placed in the agar plates and the agar allowed to set. A thin suspension of a mycobacteria culture in distilled water was then spread over the agar surface with in a ring and the moisture allowed to dry up. A tiny drop of a washed amoeba culture suspended in distilled water containing 50–100 trophozoites was then inoculated in the centre of a bacterial patch and the plates incubated at 30° C. Microscopic observations were carried out every day for a period of 10 days when subcultures were made on fresh plates containing the corresponding test organism. *Escherichia coli* which is readily eaten up by *A. castellanii* served as controls.

The results of the suitability of mycobacteria as food for *A. castellanii* is presented in Table I. A majority of mycobacteria were unsuitable as food, as evidenced by the failure of amoebae to multiply and the presence of these mycobacteria on non-nutrient agar plates in contrast to the rapid multiplication of amoebae growing on *E. coli* and clearing the bacterial patch under similar conditions. In some cases the amoebae survived without much multiplication but died out later when cultured on fresh plates indicating the possible persistence of residual culture media in the first transfer. *A. castellanii*, however, could grow and multiply when supplied with *M. kansasii*, *M. gastri*, *M. xenopi* and *M. microti* and by the third subculture were able to utilize these strains completely and encysted and excysted in a manner comparable with amoebae growing on *E. coli*.

TABLE I

Selectivity of mycobacteria as food for A. castellanii

Strains of mycobacteria	Presence of amoebae after successive subcultures*		
	First subculture	Second subculture	Third subculture
M. tuberculosis			
H_{37} Rv	+	—	—
Erdman	+	—	—
H_{37} Ra	+		—
M. bovis			
Vallee	+		—
B.C.G.	+		
M. avium	+		—
Photochromogens			
M. kansasii	++	+++	++++
M. marinum			—
Scotochromogens			
M. scrofulaceum			—
Non-photochromogens			
M. terrae			—
M. triviale			
M. gastri	++	++++	++++
M. xenopi	++	++++	++++
Rapid growers			
M. smegmatis			—
M. phlei			
M. vaccae			
M. diernhoferi	+		—
M. flavescens			—
M. chelonei			—
M. fortuitum			—
Others			
M. microti	+	++++	++++
M. ulcerans			—
Species *incertae sedes*			
M. simiae	+		
Control			
Escherichia coli	++++	++++	++++

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* Amoebae were subcultured on fresh non-nutrient agar plates containing the test species of mycobacterium at intervals of ten days.

+ — few healthy and motile amoebae present; no multiplication of amoebae.

++++ = vigorous growth of amoebae.

Singh[8] has tested a number of bacteria as food for free-living amoebae. On the basis of utilization of bacteria as food for amoebae he classified bacteria into four groups: (1) Species readily and completely eaten; (2) slowly and completely eaten; (3) those that were partially consumed and (4) an entirely inedible group. In a large number of bacterial species any particular character such as gram staining, motility, presence of proteolytic ferment, slime production among other aspects could not be correlated with their edibility or inedibility by amoebae. However, amoebae failed to grow in presence of bacteria producing toxic pigments or metabolites. In the present study similar results have been obtained when amoebae were cultured on different strains of mycobacteria. No correlation could be observed between strains that were eaten up and those that were not eaten up by the amoebae.

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---

1. Carter, R. F., *Trans. Roy. Soc. Trop. Med. Hyg.*, 1972, **66**, 193.
2. Dwivedi, J. M. and Singh, C. M., *Indian J. Microbiol.*, 1965, **5**, 31.
3. Wang, S. S. and Feldman, H. A., *The New England J. Med.*, 1967, **277**, 1174.
4. Armstrong, J. A. and Periera, M. S., *Brit. Med. J.* 1967, **1**, 212.
5. Skocil, V., Cerva, L. and Serbus, C., *J. Hyg. Epidem. Microbiol., Immunol.*, 1970, **14**, 61.
6. —, —, — and Nejeldo, V., *Cslka Epidem. Microbiol. Immunol.*, 1970, **5**, 240.
7. Proca-Ciobanu, M. and Jonescu, M. D., *Arch. Roum. Path. Exp. Microbiol.*, 1975, **34**, 329.
8. Singh, B. N., *Pathogenic and Non-pathogenic Amoebae.* Publishers: The Macmillan Press Ltd., London and Basingstoke, 1975, p. 4.

EFFECT OF IRRADIATED MEDIUM ON GROWTH AND SYNTHESIS OF MACROMOLECULES IN GERMINATING SPORES OF *B. SUBTILIS*

THE effect of irradiation has been studied in a variety of biological test systems ranging from bacteriophages to human cells which were cultured on irradiated media[1]. Irradiated bacterial medium has been shown to induce auxotrophic mutations and also retardation of growth[2–5]. Frey and Pollard[6] observed that the irradiated medium caused a cessation of DNA synthesis in *E. coli*. Contrary to the inhibitory effects, some investigators found stimulatory effects of irradiation on the germination of seeds, plants and bacterial growth[7–9]. The stimulatory effects of irradiation largely depended on irradiation dose and duration of treatment. This communication deals with the effect of gamma irradiated medium on growth and on synthesis of protein and nucleic acids in germinating spores of *B. subtilis*.

The *B. subtilis* spores were prepared in yeast extract-peptone (Y.E.P.) medium as described earlier[10]. The preliminary experiments on the growth of *B. subtilis* spores in Y.E.P. medium showed that the replication began after 4 hrs of incubation at 37° C, as evidenced by the increase in the titer of the spore concentration. The yeast extract-peptone medium was irradiated at 100 Kr using the gamma source.

About 500 ml of spore culture (containing about 10^6 spores/ml) was centrifuged at 5000 rpm and the pellet was divided into two equal halves and each was suspended in 200 ml of freshly irradiated and unirradiated media. The cultures were incubated at 37° C and stirred with a magnetic stirrer. Aliquots of 0·5 ml were withdrawn from both the cultures at various time intervals (0 to 8 hrs) and spore/cell concentrations were determined by titration on Y.E.P. agar medium plates[11].

The percentage of growth increase in irradiated and control cultures is presented in Table I. It is evident that irradiated medium at 100 Kr stimulated the growth of *B. subtilis* spores. Dharkar[8] observed stimulation of growth of several types of bacteria grown on medium containing the extract from irradiated potatoes. It was stated that stimulation of growth might occur due to a greater availability of amino acids and sugars because of radiolytic breakdown of proteins and starch or carbohydrates contained in the medium[1].

TABLE I

Growth of B. subtilis *spores in unirradiated and* 100 Kr *irradiated Y.E.P. medium*

Hours of incubation	Control medium titre/ml	% increase	Irradiated medium titre/ml	% increase
0	$35 \cdot 0 \times 10^6$	0·0	$38 \cdot 4 \times 10^6$	0·0
4	$36 \cdot 0 \times 10^6$	2·9	$164 \cdot 0 \times 10^6$	327·0
6	$52 \cdot 0 \times 10^6$	48·6	$220 \cdot 0 \times 10^6$	472·9
8	$76 \cdot 0 \times 10^6$	117·1	$344 \cdot 0 \times 10^6$	795·8

The measurements of protein, RNA and DNA were carried out in the above experiment by withdrawing samples of 10 ml from irradiated and control cultures at 0 and 8 hrs. The samples were washed twice with saline and the protein, RNA and DNA were extracted[12]. The protein estimation was done by the method of Lowry *et al.*[13]; and RNA and DNA were estimated by orcinol[14] and by diphenylamine[15] methods respectively. The contents of protein in irradiated medium culture were greater than controls (Table II) suggesting the stimulatory effect of irradiated medium on protein synthesis. Similarly, there was a stimulation in RNA and DNA synthesis in 100 Kr medium culture. Increase in RNA and DNA contents in Penicillium expansum irradiated with 50 Kr was reported by Chou *et al.*[12].

TABLE II

The measurements of protein, RNA and DNA of germinating B. subtilis *spores cultured in 100 Kr irradiated and control Y.E.P. medium*

Content	Hours after incubation	Control medium mg/10 ml culture	% increase	Irradiated medium (mg/100 ml culture)	% increase
Protein	0	10·840	0·0	11·000	0·0
	8	13·000	19·6	16·500	50·0
RNA	0	0·190	0·0	0·200	0·0
	8	0·399	110·0	0·649	224·5
DNA	0	0·130	0·0	0·140	0·0
	8	0·301	131·5	0·719	413·6

Y.E.P. medium contained 1% yeast extract, 0·8% peptone and 0·5% sodium chloride.

Thus, it may be concluded that irradiated Y.E.P. medium stimulated the growth and synthesis of macromolecules as compared to the controls.

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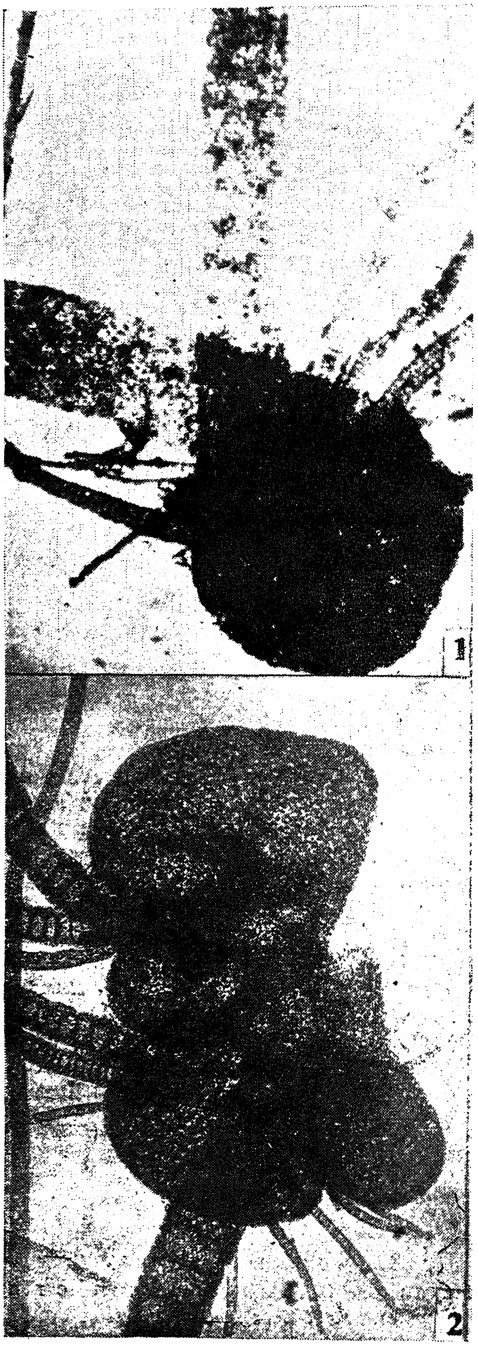
1. Keshavan, P. C. and Swaminathan, M. S., *Rad. Bot.*, 1971, **11**, 253.
2. Blank, I. H. and Arnold, W. J., *Bact.*, 1935, 30, 21.
3. Stone, W. S., Wyss, O. and Haas, F., *Proc. Natl. Acad. Sci.* (*U.S.*), 1947, **33**, 59.
4. Chopra, V. L., *Mut. Res.*, 1969, 8, 25.
5. Bhavnani, D. and Polasa, H., *Ind. J. Hered.*, 1973, **5**, 19.
6. Frey, H. E. and Pollard, E. C., *Rad. Res.*, 1966, **28**, 668.
7. Chopra, V. L., Natarajan, A. T. and Swaminathan, M. S., *Rad. Bot.*, 1963, **3**, 1.
8. Govilla, O. P., Rao, C. H. and Iyer, R. D., In *Plant Tissue and Organ Culture—Symp. Int. Soc. Plant. Morph. India*, ed. P. Maheswari and N. S. Rangaswami, 1963.
9. Dharkar, S. D., *Food Irrad. BARC* (*Trombay*), 1969, p. 27.
10. Raghavan, D. and Polasa, H., *The Ind. J. Hered.*, 1974, **6**, 109.
11. Clowes, R. C. and Hayes, W. (eds.), *Experiments in Microbial Genetics*, Blackwell Scientific Publ., Oxford, 1968.
12. Chou, T. W., Salunkhe, D. K. and Singh, B., *Rad. Bot.*, 1971, **11**, 329.
13. Lowry, O. H., Rosebrough, H. J., Farr, A. L. and Randall, R. L., *J. Biol. Chem.*, 1951, **193**, 265.
14. Pessez, M., *Bull. Soc. Chem. Biol.*, 1950, **32**, 701.
15. Burton, K., *Biochem. J.*, 1956, **62**, 315.

AN INTERESTING OBSERVATION ON A *COMPSOPOGON* GROWING AT ALLAHABAD

OUT of 11 species of the genus *Compsopogon* 5 species *viz.*, *C. coeruleus* (Belbis) Montagne, *C. hookerii*, *C. iyengarii* Krishnamurthy, *C. indicus* Das and *C. aeruginosus* (J. Ag.) Kuetzing have been reported from India[1-4]. The present note records an interesting observation on a farm growing at Allahabad which is not on record for any taxon of the genus *Compsopogon*. The alga was found growing in an uncommon habitat, *i.e.*, outlet of a continuously flowing water channel issuing from a tube-well at Allahabad. The alga grows in this habitat all round the year growing epiphytically on filaments of *Cladophora* sp. and also on bricks of the water channel and mud. Externally the thalli are olive blue green or dark blue green in colour. The thalli are coarse and profusely branched, up to 42 cm long and 2 cm broad, and are in the form of a floating mass of filaments in slow moving water. Older filaments became brittle and nodulated. The diameter of filaments at constrictions varies between 300 to 700 μ. Branches are numerous and they arise at angles from 30° to 70° with respect to the main axis. The main axis is multiseriate and normally up to 800 μ thick.

Many mature thalli show twisting and curling of the branches. At the region of twisting or curling such a filament develops a small swelling which later grows into a large discoid vegetative structure (Figs. 1 and 2). One to three such structures may occur in a filament at intervals. Later, they become still larger (600 μ–1,450 μ × 390 μ–930 μ). Such a filament breaks up at the region of vegetative discs and only one of the two fragments bears it. It appears that the vegetative disc stores food material and serves as the anchorage organ for one of the two new filaments. Thus, the discs serve as accessory structure for vegetative propagation, although monospores and microaplanospores are present for asexual reproduction. This feature is more common during the rainy season especially in the month of September. Such a feature is not recorded in any species. The taxonomic status of the present form is yet to be determined.



FIGS. 1–2. *Compsopogon* sp. Stages in the formation of vegetative disc (× 100).

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1. Krishnamurthy, V., *Phytomorphology*, 1953, 3 369.
2. —, *Ibid.*, 1957, 7, 398.
3. Patel, R. J., *Phykos*, 1969, 8, 46.
4. Pandey, R. S., Tiwari, G. L. and Pandey, D. C., *Hydrobiologia*, 1976, 3, 239.

ANTIFUNGAL ACTIVITY OF GEDUNIN

RENNERFELT[6] suggested that phenolic extractives of heartwood were more toxic in several trees of *Pinus sylvestris*; Zabel[10] indicated a correlation between decay resistance and tannin content among the trees of *Quercus alba*; Rudman and Da Costa[11] correlated variations in decay resistance of outer heartwood among trees of *Eucalyptus sieberiana* with variations in the amount of methanol soluble extractives. Recently Rudman[7–9] showed that there is direct correlation between the antifungal nature of the compound and decay resistance in several species of *Eucalyptus microcorys*, *E. triantha*, *Thuja plicata* and in sixty-four wood and bark extractives and related compounds.

In the present work the active principle of decay resistance in some of the commercial timbers, *Xylocarpus* and *Cedrela* against two wood-rot fungi, *Polyporus versicolor* L. ex. Fr. 651 (a white-rot) and *Polyporus palustris* B and C 528 (a brown—rot) is worked out.

Xylocarpus obovatus A. Juss. (Meliaceae), used in this investigation, was obtained from Andaman islands. One kg of the powdered heartwood was extracted with ten litres of petroleum ether (60° to 80°) in a soxhlet for 24 hours. The extsact was concentrated to 100 ml, when a colourless solid was obtained. This substance was purified by column chromatography over silica gel (200 gm) set up with benzene: ethyl acetate (9 : 1). The earlier fractions of the eluate gave a colourless solid (2·5 gm) which crystallised from ethanol as rhombic crystals with melting point 217–218° C. It was identified as gedunin by direct comparison with an authentic sample isolated by Akisanya *et al.*[2] and Subrahmanyam *et al.*[14].

This compound was tested for its antifungal activity by impregnating it into blocks of the susceptible wood, *Bombax ceiba*, (2·5 cm × 2·5 cm × 0·9 cm). These blocks which were depleted of extractives, if any, were oven-dried for a period of 48 hours at 105° C. Some of them were used as reference blocks while others were impregnated, according to the method suggested by Puri[5], with solutions of 0·5, 1·0, 1·5 and 2·0% gedunin using benzene as solvent. These were used as experimental test blocks and adjustment blocks.

Culture experiments determining decay resistance were conducted by soil block method (ASTM Designation—2017)[1] with modifications suggested by Bakshi *et al.*[3]. The percentage loss of weight of the test blocks and reference blocks is presented in Fig. 1. Since loss of weight in the adjustment blocks was less than 5%, it was not considered for adjustment as suggested by Bakshi *et al.*[3].

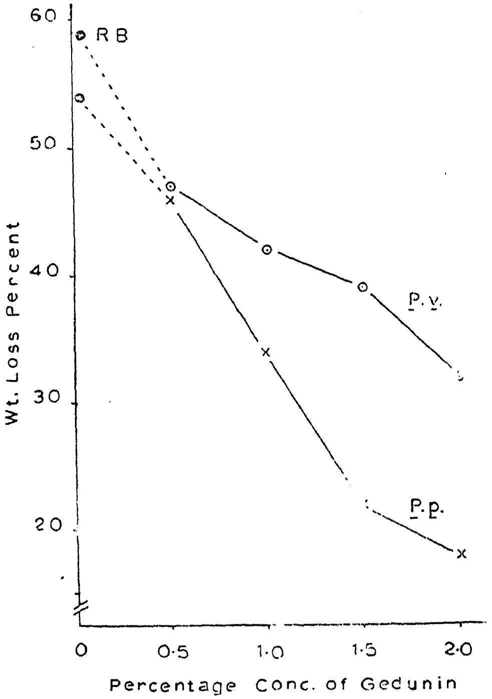

FIG. 1. Fungitoxic effect of gedunin. (P.v., *Polyporus versicolor*; P.p., *Polyporus palustris*; RB, Reference block.)

Results presented in Fig. 1 establish for the first time the identity of gedunin as one of the important extractives from the heartwood of *Xylocarpus*, demonstrating measurable antifungal activity. It is evident that gedunin is toxic to the two wood-rot fungi employed in the work, *Polyporus palustris* being more sensitive than *P. versicolor*. Gedunin showed a marked and progressive antifungal activity. The decay is 50 to 60% in the reference blocks, while it was very little in the experimental test blocks impregnated with different concentrations of gedunin.

Unextracted wood of *Xylocarpus*, which contains only 0·25% of gedunin, showed about 10 to 14% of decay (Sundarasivarao and Nazma[15], Unpublished). On the other hand the decay in experimental test blocks varied from 18 to 34% when 2% solution of gedunin was impregnated in them. It implies that, although gedunin by itself is antifungal, the greater decay resistance exhibited by the unextracted normal wood with very low gedunin content may be due to (1) the combined effect of other extractives present in the wood besides gedunin or (2) the presence of other and more stable types of toxic extractives which protect the wood from decay or (3) the probable degradation of toxicity of gedunin during the process of hot soxhlet extraction and (4) inadequate impregnation of gedunin which might have been deposited in the cell lumens and intercellular spaces in contrast to its presence in the cell walls in normal wood. Among these four alternatives the first one appears to be more convincing. This finds support from King *et al.*[4] in *Xanthoxylum flavum*; Rudman and Da Costa[11] in *Techona grandis*; Rudman[7] in *Thuja plicata*; Scheffer and Cowling[13] in *Libocedrus decurrens*; Sundarasivarao and Nazma[15] in *Cedrela toona*; all these authors have attributed the exceptional resistance to decay exhibited by these woods to the cumulative effect of all the extractives present in them.

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1. A.S.T.M. Designation, 1963, **D 2017**, 673.
2. Akisanya, A., Bevan, L. W. L., Hirst, J., Halsall T. G. and Taylor, D. A. H., *J. Chem. Soc.*, 1960, p. 3829.
3. Bakshi, B. K. *et al.*, *Final Technical Report*, F.R.I., Dehra Dun. 1967, p. 1.
4. King, F. E., Housley, T. R. and King, T. J., *J. Chem. Soc.*, 1954, p. 1392.

5. Puri, Y. N., *Indian Forester*, 1967, **93** (7), 447.
6. Rennerfelt, E., *Medd. Fran Stat. Skogs-forskningsinstitut*, 1947, **36** (9), 1.
7. Rudman, P., Plate IX, *Holzforschung*, 1962, **16** (56), 74.
8. —, Plate XI, *Ibid.*, 1963, **17** (21), 54.
9. —, Plate XVIII, *Ibid.*, 1965, **19** (52), 57.
10. — and Da Costa E. W. B., Plate II, *Forest Technological Paper*, 1958, No. 1, C.S.I.R.O., Melbourne.
11. — and —, *Jour. Wood. Sci.*, 1959, **3**, 33.
12. Savory, J. G., *Timber Papers*, Princes Risborough Laboratory, 1972, No. **50**, 1.
13. Scheffer, T. C. and Cowling E. W., *Annual Review of Phytopathology*, 1966, **4**, 147.
14. Subrahmanyam, K., Madhusudhanarao, J. and Jagannadharao, K. V., *Curr. Sci.*, 1976, **45** (8), 293.
15. Sundarasivarao, B. and Nazma, *Proc. Ind. Sci. Cong.*, 1977, Abstract No. 55, p. 74.
16. Zabel, R. A., Tech Pub. No. 68, New York State College of Forestry, Syracuse University, New York, 1948.

ABNORMAL ISOLATES OF SEED-BORNE *COLLETOTRICHUM TRUNCATUM* (SCHW.) ANDRUS AND MOORE FROM INDIA

DURING a study of seed-borne infections of *Phaseolus aureus* Roxb. (Mung bean) and *P. mungo* L. (Urid bean) from the seed samples of Haryana and Uttar Pradesh a few abnormal isolates of *C. truncatum* were isolated.

About 400 seeds of each sample were incubated on blotters and 2% P.D.A. at 20° C (± 1°) under 12 hours alternating cycles of NUV light and darkness as recommended by International Rules of Seed Testing[1]. After seven days of incubation the acervuli of the fungus were visible on the seeds as blackish irregular areas with 6–10 dark brown fillifoim setae. Minute, dull white, to almost white, conidial masses appeared intermingled be tween the acervuli. The conidia of the fungi were hyaline, falcate to lanceolate in shape. The mycelium was well developed forming sclerotial aggregates abundantly. The acervuli showed a tendency to coalesce sometimes covering the entire surface of the seeds.

On P.D.A. the colonies were dark brown to black in colour with septate branched mycelium. The individual acervuli were hemispherical to truncate conical, measuring 150 μ × 250 μ. The setae were long, 1–3 septate dark brown to almost black about 60–300 μ long and 3·5 to 8 μ wide. Immature acervuli were greyish white to dull orange in colour while matured acervuli were dark brown to black. The conidia were hyaline, falcate to lanceolate measuring 16–20 μ in length and 3·0–3·5 μ in width.

The gross morphology of the isolates was close to that of *Collerotrichum truncatum* (Schw.)[2]. However, in comparison to that species, the isolates were slow to sporulate, their conidia were at the lower end of the size range (16–20 μ in length × 3·0–3·5 μ in width; normal range 17–32 μ in length × 3·5–4·0 μ in width and the mycelium showed a greater tendency towards the formation of sclerotial aggregates. The cultures are deposited at Commonwealth Mycological Institute, Kew, England.

The authors are thankful to Dr. M. N. Gupta, Head of the Botany Department, Agra College, Agra, for providing laboratory facilities and to Dr. A. Johnston, Director, C.M.I., Kew, England, for confirming the identity of the isolates.

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1. Anonymous. "International rules for seed testing," *Proc. I.S.T.A.*, 1966, **31**, 1.
2. Arx, J.A. V., "Die Artender Gattung *Colletotrichum* Cda.," *Phytopath. Z.*, 1957, **29**, 413.

OCCURRENCE OF THREE NEW ROT DISEASES OF STORED GARLIC (*ALLIUM SATIVUM* L.)

A FEW rotten bulbs were observed in garlic from the market. The diseased garlic bulbs were yellowish-brown in colour, and light in weight as compared to the healthy bulbs. For isolation of causal organisms, the small bits of surface sterilized diseased bulbs were plated on Czapek's Dox agar media and incubated at 28° C (± 2° C) for one week. The fungi were purified following Ricker and Ricker[3] and the pathogenicity was tested by the knife injury method of Tandon and Mishra[4]. Superficial cuts were made with sterilized scalpel on surface sterilized healthy bulbs and then sprayed with spore suspension of the respective fungi. Uninjured healthy bulbs were also dipped in spore suspension for a few minutes and then incubated at 28° C (± 2° C). The pathogens were reisolated from these diseased tissues of inoculated bulbs and compared with the test organism. Corresponding controls were also maintained.

Three fungal species, *viz.*, *Cephalosporium curtipes* Saccardo, *Fusarium camptoceras* Wollen Weber and Reinking and *Penicillium paxilli* Bainier produced rots on healthy garlic bulbs through injury. It appears that these fungi are 'wound' pathogens.

All the three fungi produced dry rots which were preceded by maceration of affected tissue, shrinkage and ultimate death. *Fusarium camptoceras* caused brown rot, formation of deep fissures coupled with intensive maceration and drying of the affected tissue. The internal portion of the bulb turned complete brown with spurt of mycelial growth and the whole bulb was destroyed 12 days after inoculation. No sprouting was recorded.

Cephalosporium curtipes induced pinkish rot and two-thirds of the bulb deteriorated almost completely in 12 days time. Shrinkage of the tissue and severe maceration was evident both superficially and deeply. A little bit of sprouting was noticed which soon collapsed.

Penicillium paxilli caused darker yellowish dry rot with shallow fissures. The affected portion was soon covered with green mass. The advancement of the rot was extremely slow in terms of radial and vertical growth of mycelia but maceration and shrinkage of tissue was evident in those areas also where no fungus mycelium could be traced indicating thereby, that some toxic substance with faster diffusion capacity is involved in its pathogenesis. In twelve days time, only one-third of the bulb showed fungal growth, whereas half of the bulb was visibly affected by some diffusible toxic substance. The inoculated bulbs still exhibited considerable germination capacity but the germling finally collapsed and never reached maturity.

In terms of overall severety, *Fusarium camptoceras* was most actively involved whereas *Cephalosporium curtipes* was moderately pathogenic and *Penicillium paxilli* was least pathogenic.

Quite a good number of fungi, *viz.*, *Alternaria*, sp., *Aspergillus* sp., *Cercospora* sp., *Colletotrichum* sp., *Fusarium oxysporum* f. cepae, *Phyllosticta* sp., *Rhizopus* sp., *Pithomyces* sp., *Sclerotium* sp. and *Stemphylium* sp. have been reported to cause diseases on *Allium* spp.[1]. Recently Rangnathan *et al.*[2] have studied the varietal reaction of garlic to a new root rot caused by *Macrophomina phaseoli* (*M. phaseolina*) but so far none of the present three fungi have been reported to cause any sort of disease on garlic (*Allium sativum*) However, a white rot disease of stored garlic caused by *Sclerotium* sp. had earlier been reported from Kanpur[5].

The authors are thankful to Dr. M. N. Gupta, Professor and Head, Department of Botany, Agra College, Agra, for his interest in the present investigations.

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1. Mukerji, K. G. and Juneja, R. C., *Fungi of India*, Supplement to the list of Indian Fungi, Emkay Publication, Delhi. 1962–72.
2. Rangnathan, K., Shanmugan, M., Ramakrishnan, V. and Ramalingam, C., *Indian J. Mycol. Pl. Pathol.*, 1974, 4 (1), 93.
3. Ricker, R. J. and Ricker, R. S., *Introduction to Research on Plant Diseases*, Univ. of Wisconsin, U.S.A., 1936.
4. Tandon, R. N. and Mishra, A. N., *Indian Phytopath.*, 1969, 22, 334.
5. Thakur, R. N., Singh, B. and Lal, S. B., *Sci. Cult.*, 1962, **28** (A), 177.

OCCURRENCE OF *CYLINDROCLADIUM SCOPARIUM* MORGAN ON LUCERNE IN INDIA

During disease survey of campus flora, the authors observed the occurrence of leaf-spot and stalk blight of lucerne (*Medicago sativa* Linn.), an important poultry feed grown in the dairy farm of the University of Agricultural Sciences, Bangalore, in December 1975. Though the damage done to the plant was very little, the infection of the host became obvious with prolonged wet weather. Microscopic examination of the diseased leaflets and stalks revealed the presence of abundant cylindrical, hyaline, 2–4 septate conidia and septate conidiophores characteristic of a hyphomycetous fungus.

In nature, the disease starts on leaflets as small, circular and brown, amphigenous spots measuring up to 3 mm in diameter. On stem, the lesions were noticed at the soil level, soon enlarge, coalesce and turn necrotic. The infection appears to originate from the soil and proceeds upwards involving the stalks and leaflets.

Repeated isolations on potato-dextrose-agar medium from the diseased leaflets and dead stems invariably yielded a pure culture of a species of *Cylindrocladium*. The mycelium was septate, hyaline, cottony white at first, becoming brown with age. Older hyphae formed chlamydospores which were brown, intercalary or terminal, single or in chains, sometimes in compact sclerotium-like structure. Conidiophores were macronematous, hyaline, branching at the apex to give a penicillate appearance, erect or flexuous, cylindrical at the tip prolonging to form a sterile appendage with swollen ends.

Conidia were hyaline, smooth, cylindrical, aggregated in slimy clusters, 2–4 septate and guttulate. Based on these characteristics the organism was identified as *Cylindrocladium scoparium* Morgan.

No perithecia were observed even though it was grown on five synthetic media (2% malt agar; Martin's Rose-Bengal agar; Fomes medium; Porter Pimaricin medium and Thornton's agar) and on steam sterilized twigs of lucerne plant and incubated at 27° C for two months.

The pathogenicity of the organism was established by using 6-day-old inoculum from potato-dextrose-agar on young seedlings. Typical symptoms appeared on the leaflets after 48 hours at 95% relative humidity. Reisolation from such lesions yielded *C. scoparium.*

C. scoparium has been reported on *Ficus carica* L. (Mehta and Bose[2]), *Cajanus cajan* (L.) Millsp. (Agnihothrudu[1]), *Madhuca butyracea* (Roxb.) Macb. (=M. *indica* J. F. Gmel.) (Nirwan and Singh[3]), and *Eucalyptus macarthuri* Deane and Maiden (Pandotra, *et al.*[4]) in India.

This is, therefore, the first record of *C. scoparium* inciting leaf-spot and stalk blight of lucerne in India. The fungus grown on sterilized stem bits has been deposited in Herbarium of Agricultural College, Hebbal, as MYSP # 1985.

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1. Aginhothrudu, V., *J. Madras Univ.*, Sect. B, 1959, **29**, 155.
2. Mehta, P. R. and Bose, S. K., *Indian J. agric. Sci.*, 1947, 17, 219.
3. Nirwan, R. S. and Singh, B. B., *Indian Phytopath.*, 1967, 20, 169.
4. Pandotra, V. R., Gupta, J. H. and Sastry, K. S. M., *Sci. Cult.*, 1971, 37, 391.

RHIZOSPHERE, PHYLLOSPHERE AND GEOCARPOSPHERE STUDY OF APPARENTLY HEALTHY AND VIRUS-INFECTED GROUNDNUT PLANT

GROUNDNUT crop is generally observed to be affected with virus. As no account of rhizosphere, phyllosphere and geocarposphere microflora composition and association of useful micro-organisms with virus-infected groundnut plant has been given from this country, the present study was undertaken.

Composite samples of rhizosphere and geocarposphere soils and mature leaves from healthy and virus-infected groundnut plants at pod development stage (grown in the Biology Department) were analysed for total bacteria, *Azotobacter* and fungi by methods described by Agnihothrudu[3] Chouhan and Raina[5] and Prasad and Edward[10] respectively. The culture media used for growing of total bacteria *Azotobacter* and fungi were Thornton's (Allen and Allen[2]); Base medium '77' (Allen and Allen[2], and Czapek's Dox (Johnson *et al.*[6]), respectively. The incubation period and temperature used for growth were 4 days at ± 27° C respectively.

Predominant *Azotobacter* from the rhizosphere, phyllosphere and geocarposphere of virus-infected and uninfected healthy plants were isolated and nitrogen fixing potentiality estimated by Micro Kjeldahl Method as modified by Tripathi[15].

TABLE I

Average microbial population in groundnut sphere

Plant parts	Fungi 10^3		*Azotobacter* 10^3		Total bacteria 10^3	
	H	UH	H	UH	H	UH
Rhizosphere	45	13	141	97	1019	917
Phyllosphere	16	6	55	16	121	24
Geocarposphere	18	46	80	124	696	1940

H = Healthy plants.
UH = Unhealthy virus-infected plants.

Data furnished in Table I indicate that uninfected healthy plants of groundnut supported higher population of fungi, *Azotobacter* and total bacteria in the rhizosphere and phyllosphere over the virus-infected unhealthy plants. The increased population of healthy over diseased plants has been reported earlier in the rhizosphere (Katznelson *et al.*[7], Starkey[13], Rovira[12], and Tripathi and Edward[14]) and phyllosphere (Allen *et al.*[1]; Clark and Paul[4], Purushottam *et. al.*[11]) respectively. Contrary to the observation made on rhizosphere, Lakshmikumari[8] reported increased microbial population in the rhizosphere of tobacco mosaic virus-infected plants over normally growing healthy plants. The geocarposphere of virus-infected groundnut plants registered higher population of fungi, *Azotobacter* and total bacteria over uninfected healthy plants. Further investigations to include analysis of exudations by geocarps is suggested, in this connection with the hope of finding an answer to their anomaly, as it is possible that the geocarps of virus-infected groundnut are able to liberate more and different types of nitrogenous compounds that help to promote the multiplication of *Azotobacter*.

Data from Table II shows that *Azotobacter* associated with healthy plants had higher nitrogen fixing capacity than those associated with virus-infected plants, irrespective of plant parts from which they have been isolated. This seems to indicate that *Azotobacter* associated with diseased plants have poor nitrogen fixing capacity. Presumably this was also one of the reasons that Norris and Chapman[9] had for not recommending isolation of beneficial micro-organisms from

TABLE II

Nitrogen fixing capacity of Azotobacter (mg/g mannitol) associated from different plant parts

Plant parts Condition of plant	Rhizo-sphere	Phyllo-sphere	Geocarpo-sphere
Healthy	12·1	17·3	20·0
Virus-infected	8·6	8·6	8·9

unhealthy or diseased plants. The data in Table II also indicate that *Azotobacter* associated with geocarposphere has higher nitrogen fixing potentiality than that found in rhizosphere. This, therefore, seems to suggest clearly that different parts of the plant harbour different strains of *Azotobacter* and that geocarposphere is a potential source of desirable strains of *Azotobacter* for use as biofertilizers.

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1. Allen, L. A., Harrison, J., Watson, S. J. and Ferguson, W. S., *J. agric. Sci.*, 1937, **27**, 271.
2. Allen, O. N. and Allen, A. N., *Practical Manual in Soil Bacteriology.* Bergees Publishing Co., U.S.A., 1951.
3. Agnihothrudu, V., *Ph.D. Thesis*, University of Madras, 1954.
4. Clark, F. E. and Paul, E. A., *Advances in Agron.*, 1970, **22**, 375.
5. Chouhan, J. S. and Raina, G. L., Paper presented at 16th Annual Conference, A.M.I., Rishikesh, U.P., 1975, p. 41.
6. Johnson, F. L., Curl, Elroy, Bond, J. H. and Fribourg, A. H., Burgees Publishing Co. Minneapolis 15, Minn., 1959, p. 178.
7. Katznelson, H., Lochhead, A. G. and Timonin, M. I., *Bot. Rev.*, 1948, **14**, 543.
8. Lakshmikumari, M., *Mem. Indian Bot. Soc.*, 1960, **3**, 160.
9. Norris, J. K. and Chapman, H. M., *Identification Methods for Microbiologists—Part B.* Academic Press. Edited by Gibbs and Shapton, 1968, p. 19.
10. Prasad, J. and Edward, J. C., *Indian J. Microbiol.*, 1975, **15** (2), 121.
11. Purushottam, D., Balsundarum, C. S. and Chandramani, R., *Riso* (Italy), 1976, **25** (1), 37.
12. Rovira, A. D., *Annual Rev. Microbiol.*, 1965, p. 1965.
13. Starkey, R. L., *Bacteriol. Rev.*, 1958, **22**, 154.
14. Tripathi, S. C. and Edward, J. C., *Allahabad Farmer*, 1973, **46** (3 & 4), 273.
15. —, *D. Phil. Thesis*, University of Allahabad, 1973.

FUNGI ASSOCIATED WITH DETERIORATING SEEDS OF *CANNABIS SATIVA* L.

THE fungi associated with seed surfaces of *Cannabis sativa* L. and those invading their deeper tissues may be of some concern to the producers and manufacturers of narcotics for medicinal purposes, as they may influence not only the quality but even the quantity of the seeds. Keeping this in mind, the present investigations were taken up.

For isolation of mycoflora, nearly 400 seeds were randomly selected from 6 months old seed lot. The techniques of seed washing and agar platings were followed as suggested by ISTA (Anonymous[1]). For agar plate methods the seed surfaces were sterilized with $0 \cdot 1\%$ $HgCl_2$. The inoculated plates were incubated for a week at 20° C. The isolates were purified following Ricker and Ricker[4] and identified with the help of stock cultures of the Department and books.[2,3,5] For determining the pathogenic nature of internally borne fungi, the seeds were soaked for few hours in spore suspension and then incubated on sterilized blotting paper at 28°C. The respective fungal spores were also sprayed on leaf surfaces of 10-15 days old plants in glass house in order to determine their pathogenicity on adult crops. In all cases, care was taken to fully satisfy the Koch's postulate; corresponding controls were also maintained for which the seeds were freed from fungi by heat therapy.

From seed washing 16 fungal species were isolated, *viz.*, *Aspergillus niger* Van Tieghem, *A. flavus* Link ex Fries, *A. tamarii* Kita, *A. Sulphureus* (Fres) Wehmer, *A. repens* (Corda) de Bary, *Penicillium chrysogenum* Thom., *Alternaria tenuis* Nees, *Alternaria geophila* Daszewska, *Fusarium javanicum* Koorders, *Curvularia lunata* (Walker) Boedijn, *Cladosporium herbarum* Persoon Link, *Monilia sitophila* Sacc, *Mycelia sterilla* Cook, *Trichoderma album* Preuss, *Cephalosporium curtipes* Sacc., Streptomyces sp.

Ten fungal species were isolated from sruface sterilized seeds, *viz.*, *Aspergillus niger*, *A. sulphureus*, *Cladosporium herbarum*, *Penicillium chrysogenum*, *P. chermesinum* Biourge, *P. frequentans* Westling, *P. lavitum* Raper and Fennell, *P. fellutanum* Biourge, *P. ehrlichii* Klebahn, and *Cephalosporium curtipes*.

Five fungal species, *viz.*, *Aspergillus niger*, *A. sulphureus*, *Penicillium chrysogenum*, *Cladosporium herbarum* and *Cephalosporium curtipes* were present both externally and internally.

Out of 10 internally borne fungi only Penicillium were found to be pathogenic. The highest degree of seed spoilage was, however, exhibited by *Penicillium chrysogenum* and *P. frequentans* and the rest was moderately pathogenic. The fungi other than *Penicillium* species appear to saprophytic and probably came as a result of secondary infections. *Penicillium* *chrysogenum* also produced burning type of symptoms on leaves of adult plants and thus exhibited localised aerial infections and later caused wilting of the twig itself but never of the old plant.

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1. Anonymous, *Proc. ISTA*, 1966, **31**, 1.
2. Barnett, H. L., *Illustrated Genera of Imperfect Fungi*. Bergees Publishing Co., 426 S. Sixth Street, Minneapolis 15, Minn, (Second Edition), 1960.
3. Gilman, J. C., *A Manual of Soil Fungi*. The Iowa State College Press, Ames, Iowa, 1945.
4. Ricker, R. J. and Ricker, R. S., *Introduction to Research on Plant Diseases*. University of Wisconsin, 1936.
5. Subramanian, C. V., *Hyphomycetes*, ICAR Publication, New Delhi, 1971.

A TECHNIQUE FOR THE REVIVAL OF HERBARIUM SPECIMENS FOR FLORAL DISSECTIONS AND ANATOMICAL STUDIES

HERBARIUM specimens for floral dissections or anatomical studies are usually softened by boiling them in water or soaking in a detergent solution[1] or treating with 2·5–5·0% sodium hydroxide or sodium hypochlorite[2]. Aerosol OT solution, was sponsored as a softener for herbarium specimens[3,2]. Being a wetting agent, Aerosol functions like any similar chemical. No single technique is eqally suitable to revive all plant parts and/or species. In an attempt to devise an alternative softening agent, the present author tried for over five years, a solution of the following composition and found it to be very satisfactory in reviving herbarium specimens of various plant groups.

The mixture is made of glycerine 20 ml, glacial acetic acid 10 ml, EDTA (0·292% aqueous solution) 10 ml, sodium lauryl sulphate (5% aqueous solution) 10 ml and distilled water 50 ml. The duration of soaking the material in this solution depends on its hardness. No heating is required at any stage. The solution does not deteriorate on storage or repeated use. After the desired degree of softening the flowers can be dissected and retained in the same solution without the risk of their drying or rotting. The softened material can be returned to the herbarium sheet, after a brief washing and drying. The softened material can be sectioned free hand or on a microtome, after thoroughly washing it with distilled water. Conventional methods for microtoming and staining may be adopted. Refractory material already embedded in paraffin can be softened in this solution by slicing the wax away

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The author is t
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1. Davis, P. H. a
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2. Ayensu, E. S.,
3. Pohl, R. W., *K*

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2. *Phaeographis bicolor* Muell. Arg. *Flora* **65**: 383, 1882. Thallus thick, epiphloeodal; apothecia lirelline, immersed, short, 0·5–2·0 mm long, ends subobtuse; disc wide open, slaty; labia entire, divergent, exciple non-carbonized; ascospores 8/ascus, 5-7 loculate, brown, 6–9 × 25–40 µm in size. Chemistry: K + yellow, P + orange, stictic acid is present.

Specimens examined: Tamil Nadu, Nilgiris—73·595, 873, Palni hills, Kodaikanal—73·1985.

Distribution: Java and now India.

3. *Phaeographis dendroides* (Leight.) Muell. Arg. *Flora*, **65**: 208, 1882. Thallus smooth, epiphloeodal; apothecia lirelline, emergent, 0·5–2·0 mm long; disc wide open; exciple non-carbonized; ascospores 8/ascus, 4-7 loculate, brown, 7–10 × 20–25 µm in size. Chemistry: K +yellow, P + orange, stictic and constictic acids are present. Specimen examined: Kerala, Cardamom hills, Devicolam—76·772.

Distribution: Japan, Philippines, Sunda Islands, Sri Lanka (Ceylon) and now India.

4. *Phaeographis inconspicua* (Fee) Muell. Arg. *Memoir Soc Phys. et Hist. Nat. Geneva*, **29**: (8): 25, 1887. Thallus smooth, hypophloeodal; apothecia lirelline, semi-emergent, flexuous, 1·0–3·0 mm long; disc wide open, concave; labia cream coloured, convergent; exciple non-carbonized; ascospores 8/ascus, 4-6 loculate, brown, 5–8 × 15–22 µm in size. Chemistry: K + yellow to red, P + orange, norstictic acid is present.

Specimen examined: Kerala, Wynad forest—73·2800.

Distribution: South America and now India.

5. *Phaeographis subdividens* (Leight) Muell. Arg. *Flora*, **65**: 383, 1882. Thallus epiphloeodal; apothecia lirelline, immersed, flexuous, 0·5–4·0 mm long, ends obtuse; disc moderately open; labia entire, convergent; exciple non-carbonized; ascospores 8/ascus, 5–6 loculate, brown, 4–7 × 14–16 (22) µm in size. Chemistry: K –, P – ; No lichen substances are present.

Specimen examined: Karnataka, South Canara, Tirthahalli—74·2883.

Distribution: Sri Lanka (Ceylon) and now India.

6. *Phaeographis submaculata* Zahlbr. *Ann. Mycol.* **30**: 433, 1932. Thallus thick, epiphloeodal; apothecia lirelline, semi-emergent to distinctly emergent, 2·0–4·0 mm long, ends subacute; disc wide open, ashy white pruinose; labia entire, convergent; exciple non-carbonized; ascospores 8/ascus, 6–8 loculate, 5–7 × 20–30 µm in size. Chemistry: K + yellow to red, P + deep yellow, norstrictic acid is present.

Specimens examined: Karnataka, Coorg, Bagmandala—74·3370; Tamil Nadu, Palni hills, Kodaikanal—73·1981, 1986; Kerala, Munnar—76·472.

Distribution: China and now India.

7. *Phaeographis subtigrina* (Vain.) Zahlbr. *Cat. Lich. Univ*. **2**: 387, 1923. Thallus smooth, thick, epiphloeodal; apothecia lirelline, immersed; disc wide open, ashy white pruinose, labia entire, convergent, exciple non-carbonized; ascospores 8/ascus, 4 loculate, brown, 5–8 × 14–18 µm in size. Chemistry: K + yellow to red, P + deep yellow, norstictic acid is present.

Specimens examined: Tamil Nadu, Palni hills, Kodaikanal—73·1969, 1984, Kerala, Munnar to Kodai Road, near Yellapatti—76·622.

Distribution: Siam and now India.

8. *Phaeographina commutabilis* (Krempelh.) Zahlbr. *Cat. Lich. Univ.*, **2**: 437, 1923. Thallus thick, epiphloeodal; apothecia lirelline, black, emergent; thalline margin raised; disc narrow slit like; labia entire; black; exciple non-carbonized; ascospores 4–8 ascus, brown, muriform, 21–30 × 75–120 µm in size. Chemistry: K , P ; No lichen substances are present.

Specimen examined: Maharashtra, Mahabaleshwar—73·2931.

Distribution: Borneo and now India.

9. *Phaeographina limbata* Muell. Arg. *Journ. Linn. Soc. Bot.*, **30**: 461, 1895. Thallus smooth, hypophloedoedal; apothecia lirelline, semi-emergent to emergent, black 4·0–7·0 mm long; disc moderately open; labia thick, convergent, entire to indistinctly sulcate exciple; totally carbonized; ascospores 8/ascus, muriform, brown, 10–13 × 20–35 µm in size. Chemistry: K , P ; No lichen substances are present.

Specimens examined: Kerala, Wynad forest—74·3518, 3519.

Distribution: Africa and now India.

Grateful thanks are due to Professor M. N. Kamat, for his deep interest; Dr. Mason E. Hale, Smithsonian Institution, Washington, D.C., U.S.A., for his assistance and valuable suggestions. This work was supported by the National Science Foundation Grant (GF 36745) to the M.A.C.S. Research Institute, Poona 4.

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---

1. Culberson, C. F., *J. Chromatogr.*, 1972, **72**, 113.
2. Patwardhan, P. G. and Kulkarni, C. R., *Norw. J. Bot.*, 1977, **24** (2), 129.
3. — and —, *Biovigyanam*, 1977, **3** (in press).
4. — and Prabhu, A. V., *Curr. Sci.*, 1977, **46**, 176.
5. — and —, *Bryologist*, 1977, **80**, 348.
6. — and —, *Biovigyanam*, 1977, **3** (in press).

NEUROSECRETORY CELL TYPES IN THE BRAIN OF THE ADULT FEMALE *GRYLLOTALPA AFRICANA* (FABR.)

Introduction

IN general, two kinds of neurosecretory cells can be differentiated in the pars intercerebralis of the brain of insects by chrome-haematoxylin-phloxin staining: those that stain blue-black with chrome haematoxylin component, and those that stain red with phloxin[1]. The two types of cells are often called A– and B–cells respectively[1] Johannson[2], using paraldehyde-fuchsin, has shown for *Oncopeltus fasciatus* that the A–cells stain deep purple and the B–cells red or bluish green. On the other hand, Nayar[1], while identifying A- and B-cells by chrome-haematoxylin-phloxin staining, has reported that there is no selective staining of these cells with paraldehyde-fuchsin in *Iphita limbata*. It seems, therefore, that further studies are necessary for the purpose of comparison of cell types present in different insect species. This problem is being investigated in *Gryllotalpa africana*. The present paper describes the histology and the types of neurosecretory cells of the brain of this adult female insect.

Materials and Methods

Adult females of *Gryllotalpa africana* were used for the present histological study. The brains of these insects were removed under insect saline solution and fixed in Bouin's fluid. They were lightly stained with dilute alcoholic eosin to facilitate orientation during embedding. Paraffin sections, 6–8 μ in thickness, were stained with aldehyde fuchsin staining following the procedure given by Gomori[3] and modified by Cameron and Steele[4]. This technique demonstrates clearly the distribution of the neurosecretory colloids.

Observations

In the brain of adult insects, two distinct groups of neurosecretory cells are present in the dorsal part of the pars intercerebralis region of the protocerebrum. Each group contains about 30 neurosecretory cells (Fig. 1).

With aldehyde fuchsin staining technique, two types of neurosecretory cells are identified in each group on the basis of differences in the size and staining reactions.

The larger cells with larger nuclei (Figs. 3 and 4) stain deep purple with aldehyde fuchsin. These cells are designated as A–cells. Each group includes about 4 such cells (Fig. 3), each measuring a maximum length of 7·1 μ. The nucleus of the cell has a maximum diameter of 4·5 μ. Varying amounts of inclusions are found in the cell body and along their axons (Fig. 2).

The smaller cells with smaller nuclei stain pale purple with aldehyde fuchsin. They are referred to as B–cells. Each group contains about 25 such cells, each measuring a maximum length of 3·6. The nucleus of B-cells has a maximum diameter of 2·7 μ. The cytoplasm of these cells contains less amount of neurosecretion, as evidenced by its feeble reaction with aldehyde fuchsin (Table I). B-cells seem to vary in size (Fig. 2). Their inclusions can be traced along the axons leaving the cells.

TABLE I

Characteristics of neurosecretory cells present in the pars intercerebralis of the brain of adult female Gryllototalpa africana

Cell type	Staining reaction with aldehyde fuchsin	Degrees of secretion	Nuclear volume in μ^3
A-cell	Deep purple	***	17·96
B-cell	pale purple	*	6·23

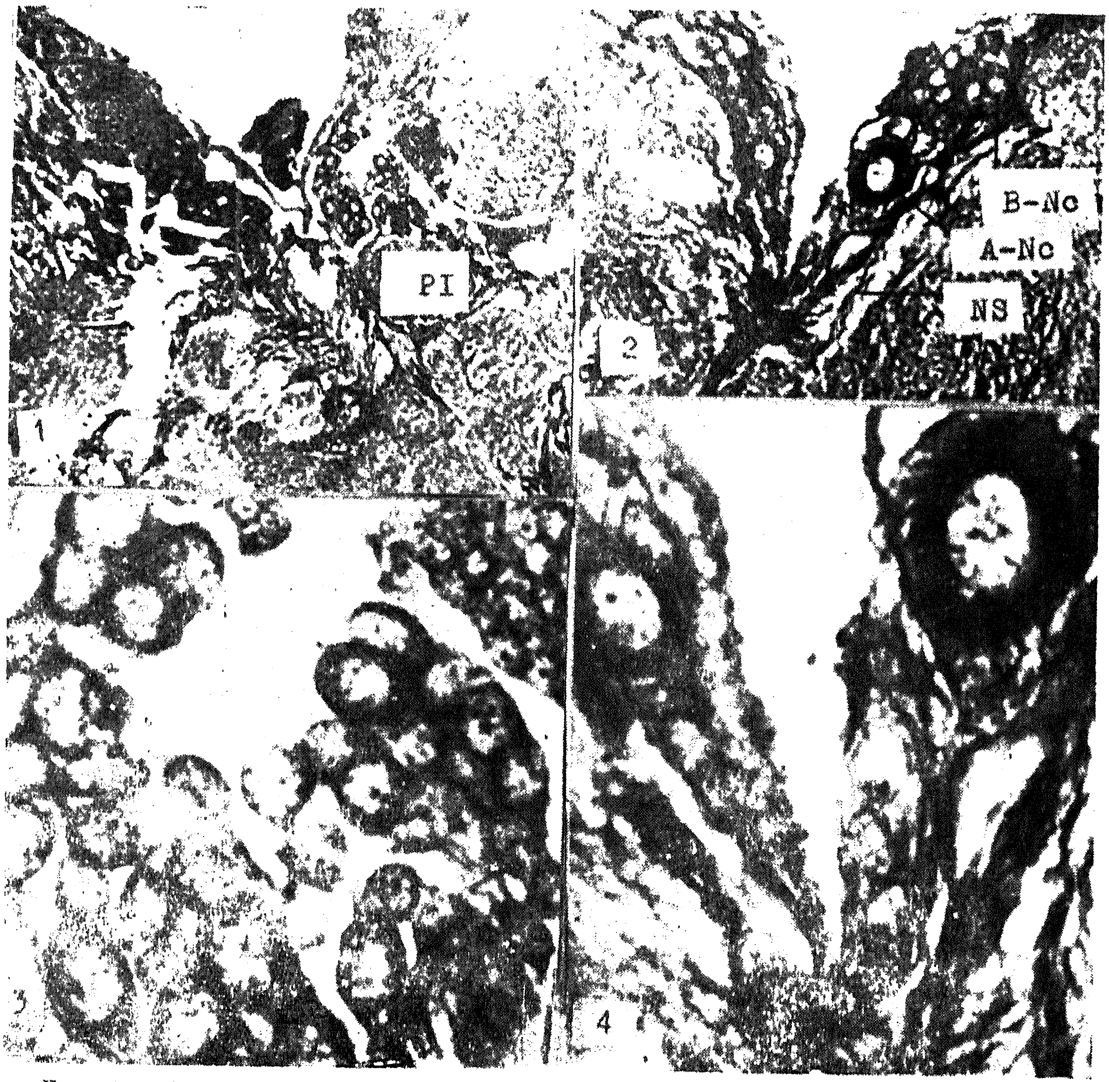
* Low concentration.

*** Very high concentration.

Discussion

The neurosecretory cells of the brain, which control many major physiological events in the post-embryonic life of insects, are generally shown to be of two kinds[5]. The present observations on *Gryllotalpa africana* have also revealed the presence of two kinds of neurosecretory cells in the pars intercerebralis part of the brain. In this insect, A- and B- cells are clearly differentiated with aldehyde fuchsin. The A-cells stain deep purple with AF and B-cells stain pale purple. These observations with reference to neurosecretory cell types of the brain are consistent with those of Nayar[1] and Johannson[2] who have reported for *Iphita limbata* and *Oncopeltus fasciatus* respectively, the existence of two types of neurosecretory cells. However, the A- and B-cells of *G. africana*, unlike those of *I. limbata*[1], show selective staining reaction with aldehyde fuchsin. On the other hand, Highnam[5], has identified, on the basis of similar staining reaction, four types of these cells, namely A, B, C and D cells, in the brain of *Schistocerca gregaria*. However, in this insect, as he has pointed out, only A- and B-cells are shown to be clearly differentiated with chrome haematoxylin-phloxin (CHP) staining. The other two kinds of cells, C and D, have been considered by him as non-secretory in nature. Highnam[5], on the basis of occurrence of axons of these cells accompnying those of A– and B-cells, has suggested that these cells (C and D) may be concerned with some mechanism which releases stored neurosecretory materials from the corpora cardiaca. On the basis of staining reaction the B-cells in *Gryllotalpa africana* appear to be similar to the C- and D- cells of the brain of *Schistocerca gregaria*[5]. On the other hand, the presence of axons of the B-cells in *Gryllotalpa africana* with stainable materials along the axon of A-cells clearly suggests that they are to be considered as a distinct cell type.

The occurrence of a higher concentration of secretory substances in the A-cells has assumed to indicate that these cells are more active than when the concentration of this substance is low[6]. On this interpretation the difference in the appearance of A- and B-cells in the mature female *Gryllotalpa africana*, with regard to the concentration of AF-positive substance, would seem to indicate that A-cells are secreting more actively than the B-cells. However, as Highnam[6] has suggested, the amount of material contained in a neurosecretory cell at any stage of development depends both upon its rate of synthesis and its rate of discharge. It, therefore, needs further investigation during different stages of post embryonic development to ascertain the secretory cycles of these cells in *Gryllotalpa africana*.



FIGS. 1–4. Fig. 1. Section of the brain showing the distribution of the neurosecretory cells into two groups in pars intercerebralis (PI), × *ca* 240. Fig. 2. Section of the brain showing A and B types of neurosecretory cells (A-Nc and B-Nc) and neurosecretion (NS) in their axons, × *ca* 370. Fig. 3. Section of the brain showing the A and B types of neurosecretory cells. Note the high and low concentration of neurosecretion in A and B cells respectively, × *ca* 1,500. Fig. 4. Section of the brain showing A-type of neurosecretory cells with larger nuclei. Note the deep staining reaction of the cytoplasm with aldehyde fuchsin, × *ca* 1,500.

The stainable material within the neurosecretory cells and corpora cardiaca is believed to be a 'carrier protein' for the actual brain hormone[5]. The presence of such stainable materials in the neurosecretory cells and axons in *Gryllotalpa africana*, thus, indicates the synthesis of the brain hormone in these cells and its transportation through the axon and NCC I (Nervous corporis cardiacum I) to the corpora cardiaca.

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1. Nayar, K. K., *Biol. Bull.*, 1955, **108**, 296.
2. Johannson, A. S., *Nytt Magasin for Zoologi*, Oslo Univ. Press, 1958, **7**, 1, pls. I–VII.
3. Gomori, G., *Amer. J. Path.*, 1941, **17**, 395.
4. Cameron, M. L. and Steele, J. E., *Stain Tech.*, 1959, **34** (5), 265.
5. Highnam, K. C., *Quart. J. Micr. Sci.*, 1961, **102** (1), 27.
6. Arvy, L. and Gabe, M., *Ann. Sci. Nat.* (*Zool.*), 1952, **14**, 345.

FOLLICULAR REGRESSION IN *TROGODERMA GRANARIUM* DUE TO STERILIZING VAPOURS OF *ACORUS CALAMUS* L. OIL

IT is a common observation that developing ovaries in insects undergo regression under the influence of chemosterilants and/or juvenile hormone analogues. Though studies in this regard are numerous[1–5], yet very few describe precisely the form and extent of regression. The present work was undertaken to study the effect of vapours of *Acorus calamus* L. (known hitherto as an inducer of male sterility in insects[6,7]) on females of *Trogoderma granarium* Everts.

Experimental

The insects were reared on semi-crushed wheat in glass jars at 27° C and 60–65% relative humidity in the culture room. The vapour treatment of *Acorus calamus* oil was given to the freshly moulted females by the method described earlier[8]. The various doses used for the treatment were 1, 3, 5, 7 and 9 μl per 60 cc of air space. Ten batches of ten insects each were taken along with the controls. The volume of the regressing ovum from fixed ovaries (10% formalin in drosophila Ringer) was measured by occular micrometer and tests were made by statistical methods.

Results and Discussion

The ovaries were fully mature at the time of emergence of the insects from their pupae and in morphology resembled those described for the species by Metwally[4]. Oviposition started 24–48 hours after emergence. The average size of the most mature ovum lying at the base of vitellarium in normal ovaries was 400 × 182 μm.

In freshly emerged insects, treatment with the vapours of *A. calamus* oil, resulted in an immediate degenerative effect in the most mature follicle and later in other parts of the ovary. The follicle got regressed in volume and the follicular epithelium autodegenerated and lost its epithelial form. This is followed by a gradual regression in the ovary and the follicular epithelium next in line. The volumetric regression in the ova of the treated insects was seen to bear a linear relationship with the dosage of the oil and the duration to which the insects are exposed (Table I).

The regression in the follicles described above cannot be compared with similar regression noticed in *Dysdercus cingulatus* under the effect of juvenile hormone analogues, because the reported regression[2] involves the immature larval ovary and not the adult ones as in the case of *T. granarium*. Moreover, JH analogue effect on adult females of *Thermobia domestica* shows[3] such regressive changes as lack of oocytes in vitellarium, failure of ovarioles to initiate yolk

TABLE I

The regressive ovum size against various doses of Acrous calamus *L. oil vapours*
(*Data on measured scale*)

Treatment		Dose (μl) 0	1	3	5	7	9
6 days	Average ovum size (μm)	400× 182	278× 176	272× 160	272× 160	240× 160	240× 112
	Volume (cμm)	3610	2100	1670	1240	761	373
12 days	Average ovum size (μm)	400× 180	210× 120	208× 112	192× 112	176× 110	160× 112
	Volume (cμm)	3602	340	280	240	200	166

deposition and in extreme cases the ovarioles become very thin and get torn; which is not the case in *A. calamus* oil treatment wherein the ovary shows most regression near the oviduct.

The reports regarding the effects by various chemosterilants like metapa and hempa at different levels of various doses are available in literature. While in lower doses (20 μg) degeneration of vitellarium as a whole has been reported[2,4], the higher doses (30 μg) induce reversible changes and any further increase in the dose invariably proves lethal. But these investitations do not locate the initial site of the effect in the treated ovaries.

In the present study the treatment for two respective groups (6 days and 12 days) revealed a normal positive correlation coefficient of 1·0 with $(x - \bar{x})$ $= 0 \cdot 0004$ and $(y - \bar{y}) = 0 \cdot 0001$ in both the cases and thus significant in both the curves (Fig. 1).

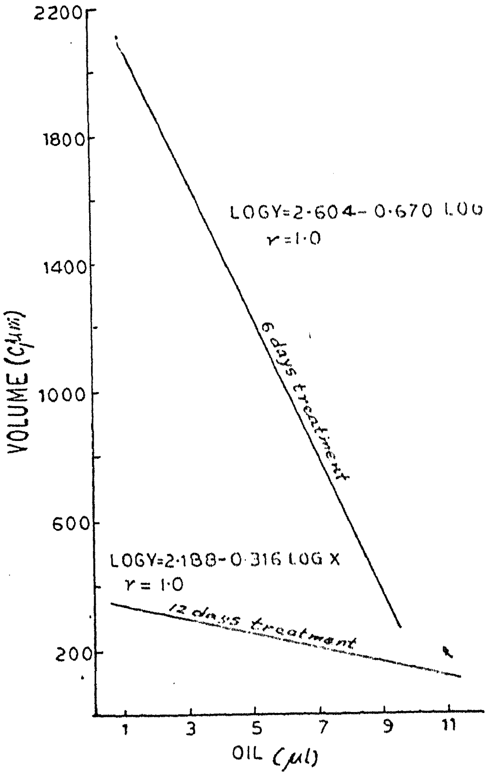

FIG. 1. Dosage response regression lines showing he relationship between dosage of the *Acorus calamus* L. oil vapours and the follicular volume.

Further, on calculating the regression coefficient or both the groups, it was found that the two relaions exhibit a gradual slope ($b = 0 \cdot 670$ and $0 \cdot 316$ espectively) although it is about 50% less in the 12-day treatment. Both correlation and regression coefficients revealed a gradual and small decrease in the follicular volume with the increase in the amount of oil and more so when the treatment time was increased.

This would, therefore, suggest that duration of exposure is probably an important factor in the treatment of *A. calamus* oil. The present finding also supports the earlier observation on *M. domestica*[9] wherein likewise a gradual overall decrease in the follicular size results with the increasing concentration of hempa.

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1. Cantwell, G. E. and Hennebery, T. J., *J. Ins. Path.*, 1963, **5**, 251.
2. Wilson, J. A. and Hays, S. B., *J. Econ. Ent.*, 1969, **62**, 690.
3. Rohdendorf, E. B. and Sehnal, F., *Experientia*, 1972, **28**, 1099.
4. Metwally, M. M., *Acta Ent. Bohemoslov.*, 1972, **69**, 229.
5. Prabhu, V. K. K. and John, M., *Ent. Exp. Appl.*, 1975, **18**, 28.
6. Mathur, A. C. and Saxena, B. P., *Die Naturwissenschaften*, 1975, **62**, 576.
7. Saxena, B. P., Tikku, K. and Koul, O., *Acta Ent. Bohemoslov*, 1977, **74** (5) (in press).
8. —, and Rohdendorf, E. B., *Experientia*, 1974, **30**, 1298.
9. Sakurai, H., *Jap. J. Saint. Zool.*, 1974, **25**, 57.

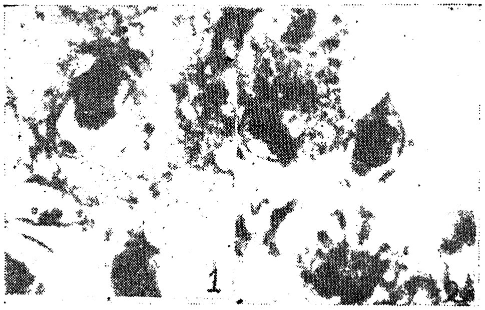
THE MIRACIDIUM OF *XENOPHARYNX SOLUS* NICOLL, 1912 PLAGIORCHIID TREMATODE FROM THE INDIAN FRESH WATER SNAKE *NATRIX PISCATOR* (SCHN)

ALTHOUGH some life cycles have been worked out in Plagiorchiidae (Yamaguti[1,2]) in respect of many species, still little is known about the miracidium, the early development in the snail intermediate host or even the nature of snail host. In a study on the biology of *Xenopharynx solus* Nicoll, 1912 collected from the gall bladder of the fresh water snake *Natrix piscator* Schn it was possible to know some features of the miracidium.

The complete development of the miracidium occurs in the uterus itself and as such the distal portion of the uterus could be seen filled with gravid eggs (Fig. 1). The eggs measure $37 \times 22\ \mu$ in size with dark amber colour.

To determine the snail host, the following species of snails (inhabiting the snake area) were used for the experiments—*Pila globosa*, *Indoplanorbis exustus*, *Alocinma travencorica*, *Melanoides tuberculatus*, *Lymnaea luteola*, *Vivipara bengalensis* and *Gyraulus convexiusculus*. One to two mature worms were teased and the eggs were exposed to snails in a petri dish and their ingestion was watched. The only snail in which the miracidia were found to be released was *G. convexiusculus*. Thirty minutes after ingestion of the eggs the snails were opened and the intestines were teased and examined. In some snails the miracidia could be seen actively moving.

The miracidium at first moves in a random way. The ciliated miracidium is more or less oval in shape with its anterior end somewhat pointed and posterior end rounded (Fig. 2). The larva measures 0·045 mm by 0·015 mm in living condition with a conspicuous gland. Minute droplets presumably coming from the apical gland could be seen issuing out at the anterior end.



FIGS. 1–2. Fig. 1. Egg with miracidium. Fig. 2. Miracidium released in the intestine of the snail *Gyraulus convexiusculus*.

Some infected snails were fixed in various fixatives and sections were taken and stained with histological stains like Azan, iron hematoxylin, etc. In sections the miracidium measures 0·025 mm by 0·015 mm. The cilia measure 0·003 mm in length. Behind the apical gland could be seen two cells with large nuclei. These cells measure 0·005 mm in diameter. Posterior to this could be seen a large densely stained body which measures 0·006 mm by 0·007 mm. This is stained dark red with the Azan technique. Posterior to this a pair of cells 0·004 mm in diameter occurs.

These miracidia swim about in the intestinal fluid until they contact the intestinal wall when they shed off the epidermal cells and penetrate.

I am indebted to Professor K. Hanumantha Rao, Head of the Department of Zoology, Andhra University, Waltair, for suggesting the problem and much useful advice.

Department of Zoology, V. VIJAYALAKSHMI.
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Waltair, *August* 18, 1977.

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1. Yamaguti, S., *Synopsis of Digenetic Trematodes of Vertebrates*. Vols. I and II, Keigaku Publishing Co., Tokyo, Japan, 1971.
2. —, *A Synoptical Review of Life Histories of Digenetic Trematodes of Vertebartes*, Kaigaku Publishing Co., Tokyo, Japan, 1975.

FLAVONOID COMPONENTS OF *MUNDULEA SUBEROSA* STEM-BARK

WE report here the isolation of two crystalline principles, identified as Sericetin (I) and Mundulone (II), from the stem-bark of *Mundulea suberosa* collected around Hyderabad, India. The chloroform extract after defattying and then chromatographing over silica gel (200 mesh) yielded I, m.p. 152° (M^+ 404), in benzene and benzene: chloroform (1 : 1) fractions. I forms a dimethyl ether on methylation and a diacetate on acetylation. II, m.p. 178–80° (M^+ 434), was obtained as colorless crystals in chloroform and chloroform: methanol eluents of the column. II formed a monoacetate. Both I and II, obtained in the present investigation have been compared (m.m.p. IR) with the authentic samples and found to be identical.

The mass spectra of I and II, hitherto not reported, showed prominent ions due to retro Diels-Alder fragmentation and generally followed the characteristic flavonoid fragmentation pattern. The occurrence of a pyreno-flavone and pyrano-isoflavone in the same source is of biogenetic interest.

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THE AGE OF THE PRESENT DESERTS OVER CENTRAL ASIA*

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IN Central Asia roughly between 37 N and 45 N and from 75 E to 110 E, there are at present vast deserts known as Takla Makan, Lop Nor, the Gobi and a few others (Fig. 4). In this area, the mean annual precipitation, as given by Lu[1], is less than 100 mm. Also, according to Watts[2], the mean maps of the Central Weather Bureau at Peking indicate a large area in Central Asia with less than an annual rainfall of 100 mm. It may also be pointed out that the mean annual precipitation recorded recently at eleven modern meteorological observatories in the Tarim Basin[2] (or Takla Makan) varies between 10 mm and 119 mm. These mean annual values are good enough to give a reasonably good picture of the present highly arid conditions over Central Asia.

From the available literature, one can state that the maximum mean rainfall occurs over these deserts in spring and early in summer. Further, according to Lange *et al.*[3], the infiltration of the rainfall over the mountain valleys in Central Asia is 40–45% in spring and autumn while in winter, most of the precipitation is lost as run-off and in summer, the precipitation evaporates away. This statement by the Russian scientists taken in conjunction with the very low rainfall figures quoted above, leads us to infer that the ground water underneath the Central Asian deserts, which could be attributed to rainfall in modern times, must be extremely small.

While the above climatological facts which are based on data of modern times can be considered as reliable, no meteorological evidence has so far been adduced in support of the following archaeological findings which can also be considered as well-established.

(*a*) There were Palaeolithic and Neolithic settlements[4,6] in these areas.

(*b*) Relics of Chinese art and civilisation in the pre-Buddhist period, dating back to the second century B.C., have also been unearthed[4,5] in these desolate regions.

(*c*) Buddhist frescoes and some of the world's most moving sculptures of the Buddha dating back to the period from the second century A.D. to the eighth or ninth century A.D. have been found[4,5] in these deserts.

(*d*) There are at present numerous dried-up river-beds[4,5] in these deserts. However, "the water table is high and water may often be found within 20 feet of the surface"[6].

(*e*) Archaeologists and others[4,6] have repeatedly stressed that there has been general desiccation over the whole of Central Asia during *historical times*. It has also been established that some of the famous Buddhist sites, *e.g.*, Niya (38° 00′ N, 82° 45′ E) and Miran (38° 16′ N, 89° 00′ E) were abandoned by the inhabitants "about or soon after the close of the third century A.D."[4].

The following paragraphs are a preliminary attempt by the author to adduce meteorological evidence in support of the above findings.

Lamb, Lewis and Woodroffe[7] have recently published the 1000–500 mb. mean thickness patterns** for July around 6500, 4000, 2000 and 500 B.C. epochs and for modern times (1949–1957). The patterns for the pre-historic periods have been derived largely from palaeobotanical and radiocarbon dating evidence. According to Lamb *et al.*[7], the thickness patterns can be taken to determine the shape of the circumpolar vortex of upper westerly winds. In support of this view and of their special usefulness in climatic studies, see Sutcliffe[8] and Lamb[9]. Figs. 1, 2, 3 and 4 show the thickness patterns in July for 6500, 4000, and 500 B.C. epochs and for modern times respectively.

On the diagrams of Lamb *et al.*, the present author has drawn the trough-lines of the major troughs between 30 E and 120 W. The errors in our determination of the positions of the troughs may be five degrees or more in longitude but this degree of accuracy is good enough for the purpose we have in view. The other major troughs seen in the diagrams are obviously too far away from Central Asia to affect directly this area.

A careful examination of the thickness patterns, keeping in view the limitations of our analysis, shows the following :

In all the 4 patterns for the pre-historic epochs 6500, 4000, 2000 (not reproduced here) and 500 B.C.

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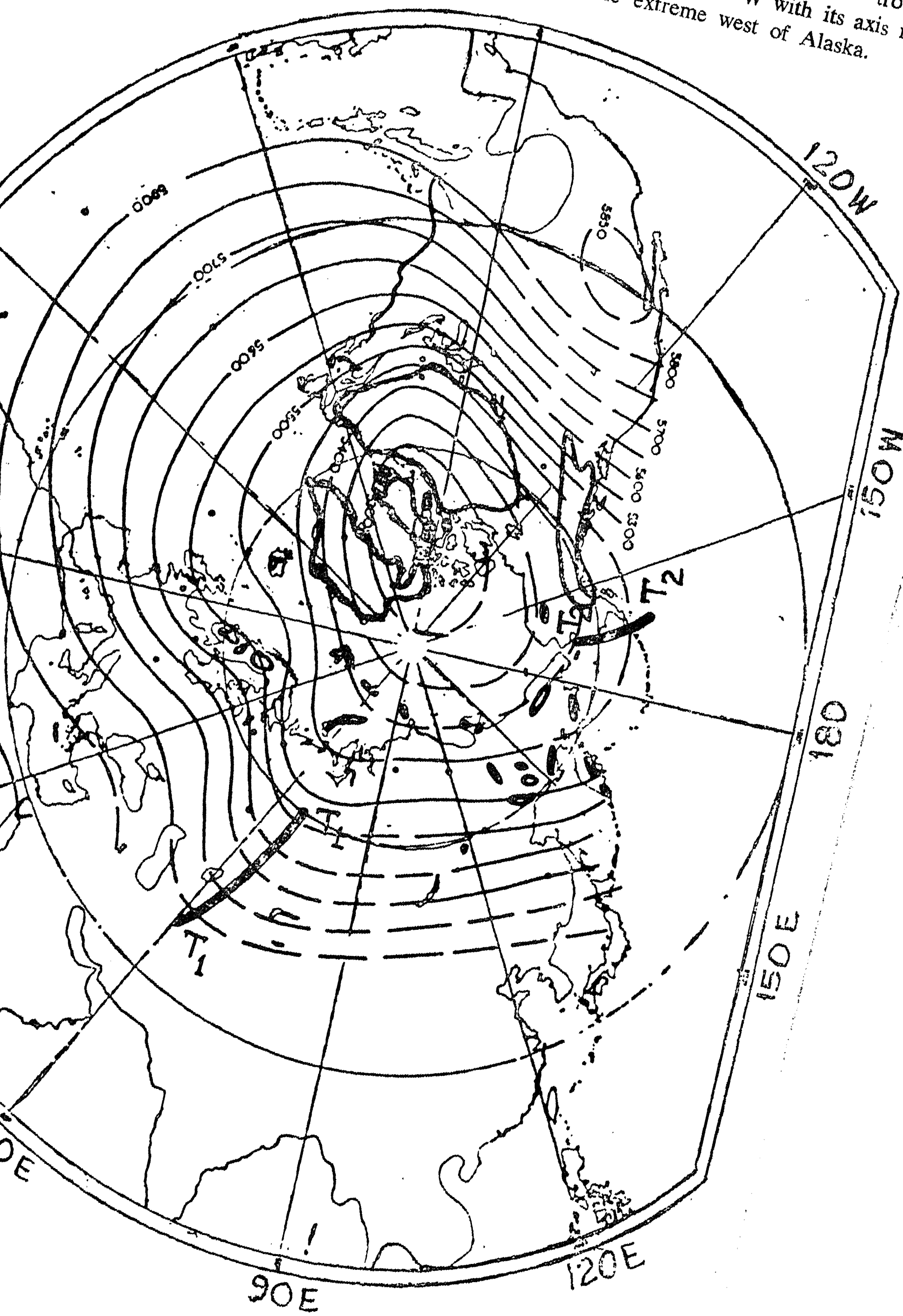
* Based on a lecture delivered by the author under the auspices of the Indian Meteorological Society, Delhi, on 19th July 1977.

** In the area where the authors have drawn the patterns as dashed lines, we may assume that the lines have been considered by them as tentative.

(a) there is a major trough (T_1T_1—see Figs. 1, and 3) with its axis between 60 E and 90 E, *i.e.*, r about 70 E to the west of Lake Balkash.

(b) there is another major trough (T_2T_2) between 180° and 150 W with its axis running over or near the extreme west of Alaska.



Per contra, in the patterns for modern times (Fig. 4),

(*a*) there is no trough anywhere between 60 E and 90 E which we had observed in the pre-historic epochs. Instead, we see a major trough T_3T_3 between 150 E and 180°, *i.e.*, near 165 E with its axis to the east of Kamchatka in USSR ;

(*b*) there is also a major trough T_4T_4 between 150 W and 120 W, *i.e.*, off the west coast of Canada and U.S.A.

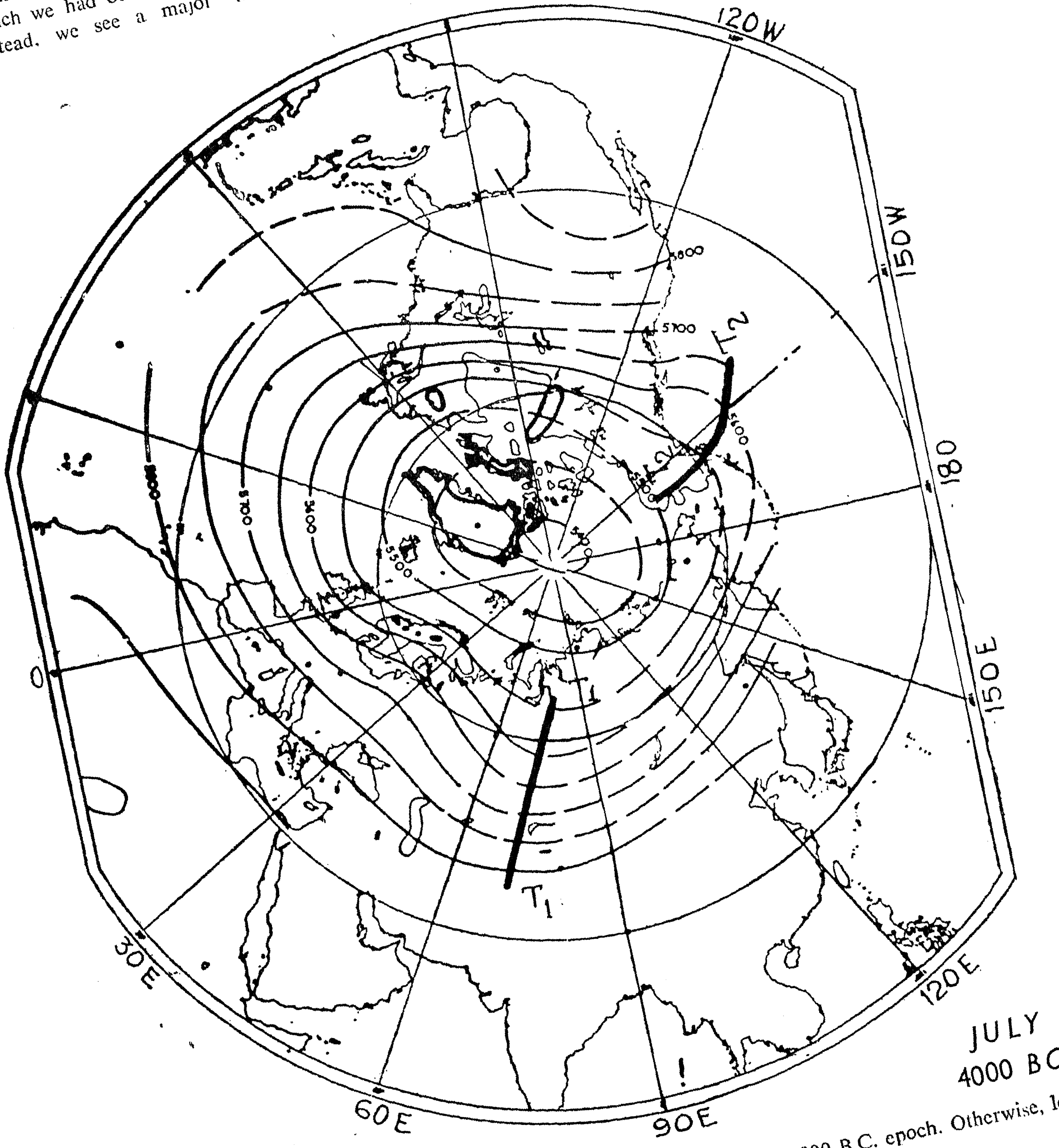


FIG. 2. Mean 1000–500 mb. thickness patterns in July during 4000 B.C. epoch. Otherwise, l
ame as for Fig. 1.

We have the following two possible explanations for the observed positions of T_1T_1 and T_2T_2 in relation to T_3T_3 and Γ_4T_4 :

Explanation-1 : T_1T_1 may have shifted eastwards to the position T_3T_3 between 500 B.C. and the 1950's. Likewise, during the same period, the trough may have shifted eastwards to the position Our climatological and synoptic experience that the shifts of these mean troughs must been gradual and not abrupt.

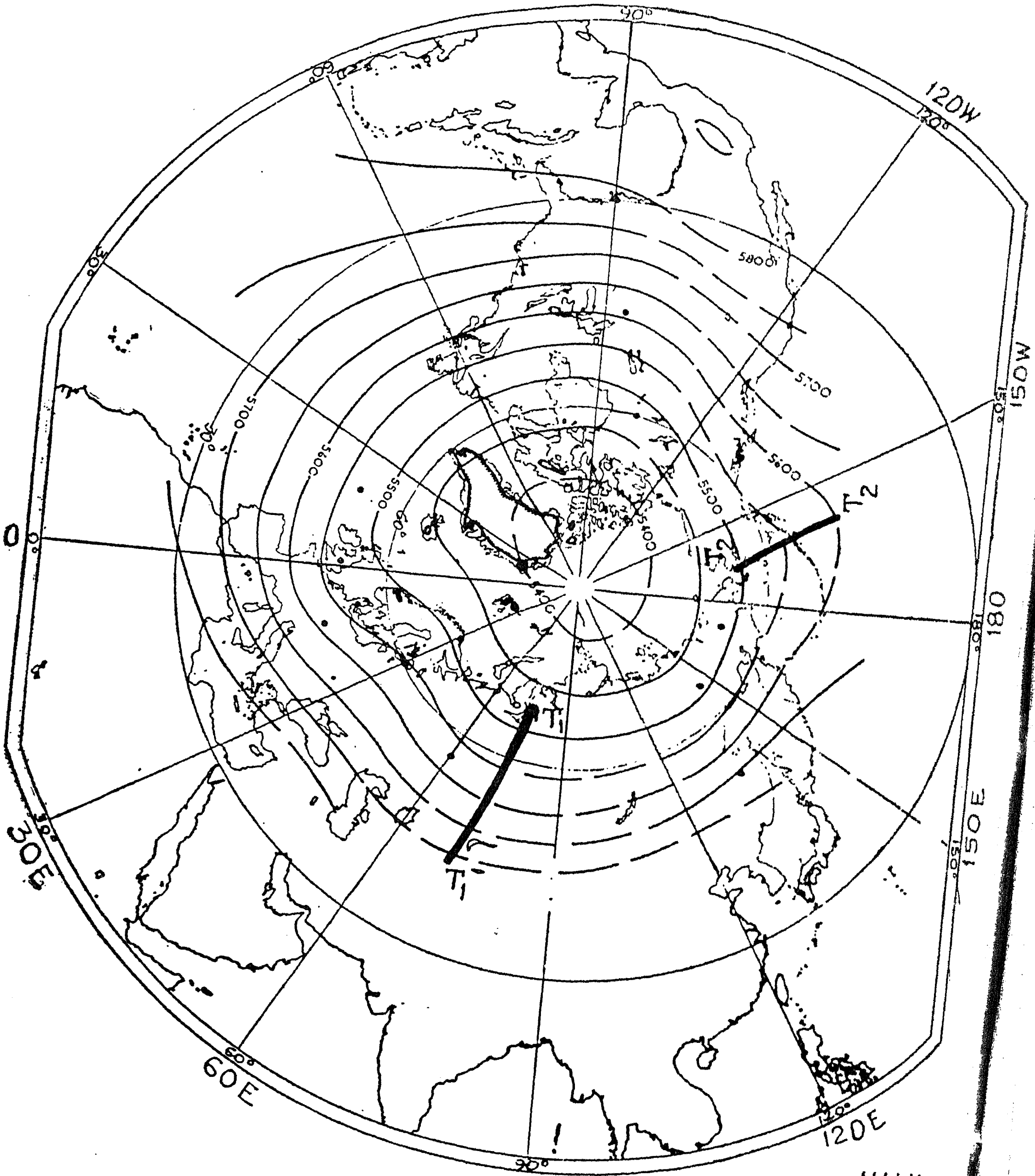


FIG. 3. Mean 1000–500 mb. thickness pattern in July during 500 B.C. epoch. Otherwise, legend same as for Fig. 1.

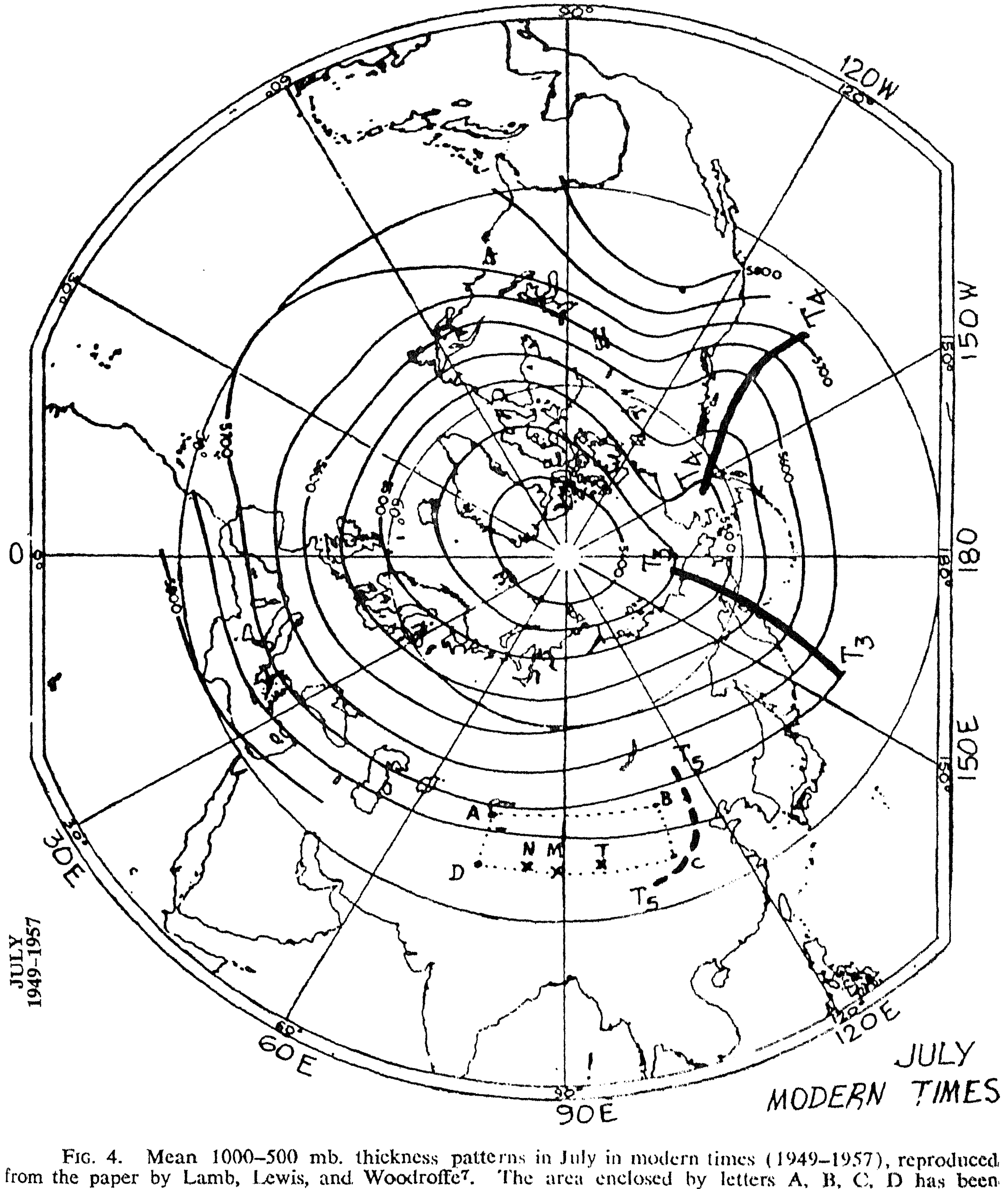


FIG. 4. Mean 1000–500 mb. thickness patterns in July in modern times (1949–1957), reproduced from the paper by Lamb, Lewis, and Woodroffe[7]. The area enclosed by letters A, B, C, D has been demarcated by the present writer. It shows roughly the area covered by the deserts over Central Asia. Letter N stands for Niya and M stands for Miran, the two famous Buddhist sites referred to in the text. 'T' stands for Tun–Huang where the Caves of the Thousand Buddhas are situated. Trough lines T_3T_3, T_4T_4 and T_5T_5 have been drawn by the present author. Note that the trough T_1T_1 which is so conspicuous in Figs. 1, 2 and 3 between 60 E and 90 E is completely absent in this diagram. T_5T_5 (thick dashed line) is the axis of the trough south of 50 N in the mean 500 mb. chart in modern times for July as published by Academia Sinica, Peking[10]. Note that the Central Asian deserts lie "in the rear" of the modern trough T_5T_5.

Explanation–2 : T_1T_1 and T_2T_2 may not be related in any way to T_3T_3 and T_4T_4. The former may have disappeared after the pre-historic period and T_3T_3 and T_4T_4 may have developed *in situ* in modern times.

Of the above two explanations, No. 1 seems to be much more plausible as T_1T_1 and T_2T_2 have both shown an eastward shift although the eastward shift of T_1T_1 is rather high compared to that of T_2T_2. But irrespective of explanation–1 or explanation–2, *an irrefutable fact emerges from our analysis,* viz., *the regions which are now deserts in Central Asia lay "ahead of" the trough T_1T_1* (i.e., *to the east of the trough*) *in the pre-historic epochs but they are not so in modern times.* On the contrary, they are situated to the west of a trough (T_3T_3) during the latter period. T_3T_3 is however rather far away from the Central Asian deserts to justify the statement in meteorological parlance that they lie "in the rear" of the trough. The reason for this rather distant position of T_3T_3 will be explained in a later paragraph. We would merely state at this stage that the mean 500 mb. chart for July (the complete diagram is not reproduced here due to considerations of economy of space) published by the Staff Members of Academia Sinica, Peking, shows two troughs. One of these lies with its axis between 110 E and 115 E. This has been shown as a thick dashed line T_5T_5 in Fig. 4. The second trough in the Peking charts lies close to Kamchatka, *i.e.*, almost along the same longitude as T_3T_3 published by Lamb *et al.*, and as seen in Fig. 4. The mean 500 mb. chart of Academia Sinica, according to the Chinese authors, is based on a very large number of modern upper air stations and is therefore considered as very reliable. We are aware that we are comparing a mean 500 mb. contour chart with a mean 1000–500 mb. thickness chart. This does not however introduce any major error in our analysis as we are dealing with broad climatic features of the general circulation of the atmosphere. The fact that both the 1000–500 mb. thickness chart of Lamb *et al.*, and the 500 mb. contour chart of Academia Sinica show the Kamchatka trough almost in an identical position, lends further support to our view. We can therefore legitimately infer that the diagram published by Lamb *et al.*, does not show T_5T_5 because their mean was based on inadequate data over Central Asia which was not the case with the chart published by Academia Sinica. We can accordingly accept that T_5T_5 is a genuine mean 500 mb. trough over Central Asia in modern times. If we accept this view, we immediately see that there is a greater consistency in the amount of eastward shift of the troughs T_1T_1 and T_2T_2 from 500 B.C. to modern times. It also becomes obvious that the Central Asian deserts *lie in modern times "in the rear" of trough T_5T_5 (Fig. 4) in modern times.*

In view of what has been stated above, we can confidently say that the ancient trough T_1T_1 around 6500 B.C., 4000 B.C., 2000 B.C and 500 B.C. would have, in individual synoptic situations, caused frequent and fairly large amount of rainfall over Central Asia between 6500 and 500 B.C., *Per contra* in the same area of Central Asia, in modern times, there would be subsidence, inhibition of convection and development of arid conditions in the rear of trough T_5T_5 (Fig. 4). If our above line of reasoning is correct, we should also expect a sharp increase in normal rainfall "ahead of" trough T_5T_5 which, it may be recalled, relates to modern times. That it is actually so can be seen from the normal rainfall in modern times published by Watts[2]. For instance, along the latitude range 39 N–40 N, the annual rainfall increases from 204 mm at Patou (110 E) to 623 mm at Peking (116 E) and to 1308 mm at Wonsan (127 E). Similar increase takes place in rainfall in July also.

Now, if we accept that explanation No. 1 given in an earlier paragraph is much more plausible, the progressive eastward movement of T_1T_1 would lead to a corresponding progressive eastward extension of the desert area in the rear of trough T_1T_1 (due to subsidence, inhibition of convection and consequent absence of rainfall) as we approached modern times. Such gradual eastward extension of the desert (*i.e.*, of the area where human habitation would have become progressively more and more difficult). is supported by the archaeological evidence pointed out by us earlier about the abandonment of the Buddhist sites at Niya (83 E) and Miran (89 E) "about or soon after the close of the 3rd century A.D."[4]. Likewise, it would be supported by the archaeological evidence regarding the cave-sculptures of the Thousand Buddhas near Tun–Huang (95 E), carved out during the T'ang Dynasty[4] (618 A.D. to 907 A.D.[11]). It is unthinkable that the T'ang emperors would have created such wonderful sculptures of the Buddha in a lifeless desert. In other words, the axis of the mean trough T_1T_1 would have been to the *west* of Tun–Huang (95 E) when the Thousand Buddhas were carved out by the T'ang emperors.

The frequent and fairly large amount of rainfall over Central Asia ahead of the Trough T_1T_1 between 6500 and 500 B.C. would have infiltrated into the soil for about six thousand years. As the ground water would not have been exploited during this long period, it is not unreasonable to expect that it would have gone on accumulating underground.

As we do not however have an idea of the geological formation underneath these deserts, we are unable to state how much of the accumulated ground water would have flowed away. Nevertheless, one could broadly expect from our line of reasoning that the pre-historic ground water is still underneath these deserts and that consequently, the water table is high as we see it today[6].

On the basis of the above facts and arguments :he author would put forward a tentative hypothesis hat *Central Asia was not a desert region between 5500 B.C. and 500 B.C. and that deserts began to levelop only after 500 B.C.* Thereafter, they ;radually extended eastwards. This eastward xtension would have continued at least upto the nd of the T'ang Dynasty. The water table must herefore have risen nearly to its present height by ie end of the T'ang Dynasty. The subsequent ccumulation of ground water must have been small. he correctness of this statement about the age of ie ground water can be checked by C_{14} tests as : the case of the ground water reserves undereath the Thar Desert[12] in India. It is relevant to ld that the age of the ground water determined y C_{14} tests may be out by about one thousand† :ars, but even then, the upper limit of the age of e ground water underneath the Central Asian serts would be at least six thousand years.

The author would express his thanks to of. Y. P. Rao, Director-General of Observatories, ew Delhi, for the facilities provided to him, for s investigation.

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† Recent communication to the writer from the tional Centre of the Government of India for iclear Sciences and Mathematics (TIFR), mbay.

1. Lu, A., *Collected Sci. Papers (Meteorol.) Acad. Sinica, Peking*, 1954, p. 467.
2. Watts, I. E. M., *World Survey of Climatology*, (edited by Arakawa, H), Elsevier Publishing Company (Amsterdam, London, New York), 1969, 8, 1.
3. Lange, O., Ivanova, M. and Lebedeva, N., *General Geology*, Foreign Languages Publishing House, Moscow, p. 159.
4. Stein Aurel, M., *Innermost Asia*, 1928, I and II, 85 (Clarendon Press); *Ruins of Desert Cathay*, 1912, I, 225 (Macmillan); *Ruins of Desert Cathay*, 1912, II, 55 (Macmillan).
5. Milfred Cable, and Frenecsca French, *Gobi Desert* (Hodder and Stroughton), 1943, p. 14.
6. *Encyclopaedia Brittanica*, 1956, 10, 458; 1956, 21, 815; 1971, 10, 511.
7. Lamb, H. H., Lewis, R. P. W. and Woodroffe, A., *World Climates from 8000 to 0 B.C.—Proc. International Symp. Royal Meteorol. Soc.*, 1966, p. 198.
8. Sutcliffe, R. C., *Quart. J. Roy. Meteorol. Soc.*, 1947, 73, 370; *Met. Mag. London*, 1948, 77, 147.
9. Lamb, H. H., *Phil. Trans. R. Soc. London*, 1974, A 276, 217.
10. Staff Members, Academia Sinica, Peking, *Tellus*, 1957, 9, 432, 433.
11. Schafer, H. Edward, and Editors of Time-Life Books, *Ancient China*, Great ages of man, Time-Life International (Nederland), 1970, p. 185.
12. Ramaswamy, C., *Nature*, 1968, 217, 628; *International Hydrological Decade Newsletter, India*, 1972, 17, Appendix 4.

STUDIES ON THE RELATIONSHIP OF CINEOLE CONTENT AND REFRACTIVE INDEX OF *EUCALYPTUS* HYBRID OIL

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ABSTRACT

An attempt to establish a relationship between refractive index and cineole content of the essential oil of *Eucalyptus* hybrid (Mysore gum; mainly *E. tereticornis*) was made. It was observed that oil samples having refractive indices less than 1·4700 were rich in cineole.

INTRODUCTION

RGE scale plantations of *Eucalyptus* hybrid (Mysore gum; mainly *E. tereticornis*) have been undern in Karnataka and other States as a raw material pulp industry. The leaves go to waste when the is extracted. Some of the *Eucalyptus* hybrid trees yield oil comparable in cineole content to the oil obtained from *Eucalyptus globulus* (Blue gum) and the significance of *Eucalyptus* hybrid as an alternative source of cineole was suggested earlier[1]. A screening of these trees was taken up with a view to identifying cineole-rich trees, which are morphologically indistinguishable. Though for the rapid initial identification of such trees the olfactory test could be used, yet for the actual identification the oil obtained from the leaves needs a quantitative examination for cineole content by chemical method.

Since the chemical method is time-consuming and requires 3 g of oil for each estimation, large amount of leaf material has to be obtained. It also involves the use of O-cresol which is hygroscopic and the presence of moisture affects the results. With a view to finding a simpler technique, the percentage of cineole in the oil and its specific gravity, optical rotation and refractive index were determined for establishing possible correlation.

MATERIALS AND METHOD

Leaf samples were collected using olfactory test from 315 trees apparently rich in cineole. The essential oil was obtained from the leaf samples by steam distillation and the physical constants, *viz.*, specific gravity, optical rotation and refractive index were determined after the oil was dried with anhydrous sodium sulphate overnight. The Abbe's refractometer was used to determine the refractive index. The cineole content of each sample was estimated by O-cresol method[2]. From the population of these data, 15 samples were selected by random sampling method and correlation studies were made between the cineole content and physical constants.

RESULTS AND DISCUSSION

The data pertaining to the 15 samples selected are presented in Table I. The correlation coefficient obtained between cineole content and various physical constants of the *Eucalyptus* hybrid oil are given in Table II.

From Table II, it is clear that there is a significant correlation between cineole content and refractive index indicating an association between the two variables. It was observed that all the samples with refractive index below 1·4700 were cineole rich.

Due to the high degree of association between cineole content and refractive index an attempt to fit a linear regression line was made. The best equation was found to be

$$Y = 2640\cdot9025 - 1760\cdot25\,X \quad \ldots \quad (A)$$

Standard error of the regression coefficient (Sb) = 600·7 and this has been shown in Fig. 1.

For any given refractive index (X) its experimentally determined value of cineole content (Y) are scattered above and below the regression line given by equation A. In practice, it is of interest to know how closely the regression could predict the individual value of cineole content rather than the mean value obtained by the use of equation A. Hence the range of variation was determined using 95% prediction interval[3]. The prediction interval is given by the equation

$$Y' = 2640\cdot9025 - 1760\cdot25\,X \pm 10\cdot54\,[1\cdot07 + 5000\,(X - 1\cdot4661)^2]^{\frac{1}{2}} \ldots (B)$$

where X is the known refractive index. Estimated prediction interval is shown by the dotted lines in Fig. 1.

The reliability of the estimates obtained by the use of equation (B) was tested on samples obtained from a different plantation. The estimates obtained by the equation and also by the experimental method were well within the 95% prediction interval, indicating a good reliability of the estimate by the equation.

TABLE I

Data of 15 random samples considered for correlation studies

Cineole content %	Physical constants (at 27° C)		
	Refractive index	Specific gravity	Optical rotation
71·8	1·4605	0·9181	3·90°
69·9	1·4620	0·9103	2·30°
68·8	1·4640	0·9279	2·7°
63·8	1·4655	0·9122	1·2°
62·5	1·4670	0·9122	−22·7°
59·9	1·4635	0·9189	7·8°
59·5	1·4650	0·9088	−23·2°
57·6	1·4660	0·9279	3·00°
56·9	1·4690	0·9217	−26·55°
56·3	1·4650	0·9103	−26°
56·1	1·4705	0·9181	− 1·8°
52·8	1·4690	0·9189	− 2·00°
51·8	1·4700	0·9088	− 7·9°
51·5	1·4700	0·9103	−12·1°

TABLE II

Correlation coefficient between cineole content and its physical constants of Eucalyptus *hybrid oil*

	Physical constants		
	Refractive index	Specific gravity	Optical rotation
Cineole content	−0·840*	0·181	0·286

* Significant at 1% level.

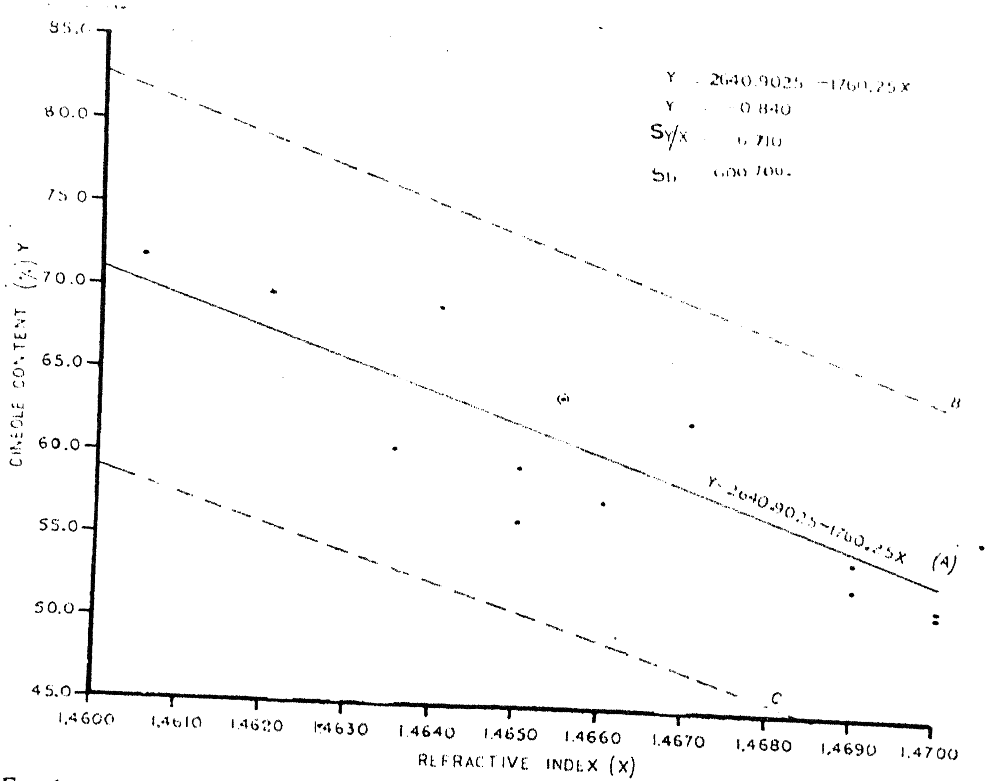

FIG. 1. Relationship of refractive index to cineole content (Dashed lines B & C show 95% prediction intervals).

ACKNOWLEDGEMENT

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1. Theagarajan, K. S. and Rao, P. S., *Ind. Forester*, 1970, **96**, 347.
2. Guenther, E., *The Essential Oils*, Vol. I (D. Van Nostrand Co., New York), 1961, p. 295.
3. Crow, B. L., Davis, F. A. and Maxfield, M. W., *Statistics Manual* (Dover Publications Inc., New York), 1960, p. 163.

INTERNATIONAL ORGANIZATION OF OLIGOCHAETE TAXONOMISTS

At an international meeting of oligochaetologists held in Braunschweig 28–30 June, 1976, it was agreed to found the International Organization of Oligochaete Taxonomists under the presidentship of Dr. A. Zicsi (Budapest) to promote co-operation and the exchange of information among research workers on all aspects of oligochaetology and to initiate the preparation of any checklists deemed to be required. Membership is open to all interested workers; for details apply to: Mr. B. W. Sims, Department of Zoology, British Museum (Natural History), Cromwell Road, London, SW 7, 5 BD.

ON THE OCCURRENCE OF STROMATOLITES IN THE KROL FORMATION OF NAINITAL AREA AND ITS IMPLICATIONS ON THE AGE OF KROL FORMATION

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ABSTRACT

A well-developed stromatolite assemblage of *Conophyton garganicus–Baicalia baicalica–Colonella* sp. is recorded for the first time from the upper Krol sediments of Nainital area. This is a characteristic assemblage of Proterozoic (middle Riphean) in India, U.S.S.R. and Australia. The age of Krol is thus late Precambrian, and is correlated with calc zone of Pithoragarh, Shali limestone, Deoban limestone, Vindhyan and Aravalli sediments. The stromatolites of Krol sediments are also associated with phosphorite and galena mineralization.

INTRODUCTION

KROL formation is an important lithostratigraphic unit of the lesser Himalaya, and is made up of essentially limestone and dolomite. Medlicott[1] proposed the name Krol formation for the sequence of dolomite, limestone, shale, and marl of the Krol hill near Solan, which was later investigated in detail by Pilgrim and West[2], Auden[3] and others. Krol succession in Nainital area makes a major syncline and represents south-eastern extremity of Auden's Krol belt, and was first studied by Middlemiss[4]. Later Pal and Merh[5], Pande[6], Fuchs and Sinha[7] made detailed stratigraphic and structural studies in Nainital area.

During the course of a sedimentological study of Krol formation of Nainital area, some well-developed stromatolites have been recorded in the upper Krol dolomites of Nainital area. This paper presents data on these stromatolites.

KROL FORMATION OF NAINITAL AREA

Krol sediments in Nainital area are 700–800 m thick; the upper Krol sediments (Krol C, D and E of Auden) represent dominantly algal mat deposits, together with oolitic limestone, tidal flat succession of sandstone and shale. Algal mats and branching stromatolites are recognized in these sediments by Fuchs and Sinha[7]. The algal mat deposits of Krol sediments show algal mats with smooth laminations, algal mats with contorted and corrugated laminations, algal mats with fenestral structure, loferite, oncolite, dolomite, bird's eye dolomite. These facies represent deposition in a protected bay in intertidal to supratidal zone. The facies is comparable to algal mat facies of Shark bay (Davies[8]). Associated with algal mat deposits are also a few horizons with well-developed columnar stromatolites. In this paper two of such horizons are discussed.

(A) *Horizon of* Conophyton garganicus *and* Colonella *sp.*

Within a sequence of black limestone showing algal balls, oncolites, algal mats, there are horizons showing *Conophyton garganicus* and *Colonella* sp. There are *ca.* 50 cm thick horizons made up of *Colonella* sp. in which laminae are continuous. Within this unit *Conophyton garganicus* is developed. These thicker limestone units alternate with 5–15 cm thick shaly limestone units made up of small *Colonella* sp. colonies.

SYSTEMATIC DESCRIPTION

Supergroup—Conophytonida, Group—*Conophyton*, Form—*Conophyton garganicus* Koroljuk, 1963 (Figs. 1, 2).

Colonies are conical to subcylindrical (height 10–25 cm and diameter 4–8 cm) showing conical internal laminae. Circular to elliptical in cross-section. In longitudinal section a prominent axial zone is developed, where the thickness of dark laminae is distorted, while the thickness of light bands remain relatively constant. The dark laminae are smooth to lenticular. Within the laminae galena is present.

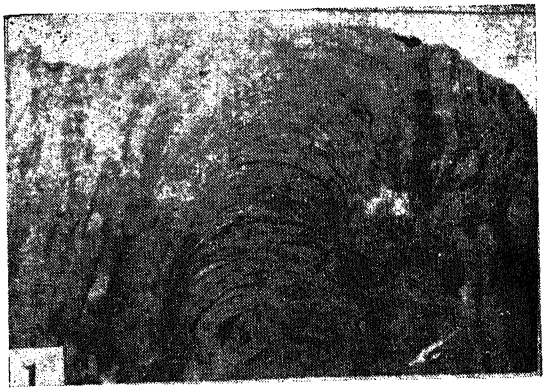
(B) *Horizon of* Baicalia baicalica

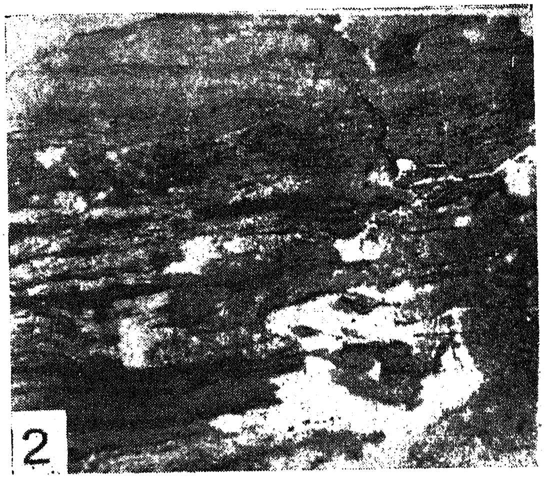
This is a 1 m thick horizon of stromatolitic limestone showing dark coloured elongated stromatolites showing branching characteristics, and abundant oncolites. It is underlain by a calcareous shale showing tidal bedding, and overlain by algal mat dolomite, showing faintly crenulated laminae with well-developed lamellar fenestrae.

SYSTEMATIC DESCRIPTION

Supergroup—Tungussida, Group—*Baicalia*, Form—*Baicalia baicalica* Krylov (Figs. 3, 4).

Colonies tuberous, making angle of a few degrees to 90° with the horizontal. Height 10–20 cm, width 2–6 cm, branching in the form of dichotomous thinner columns. Margins of the column uneven or rugged. Laminae are non-enveloping, convexity of the laminae moderate to flat. Boundaries of dark laminae are gradational.



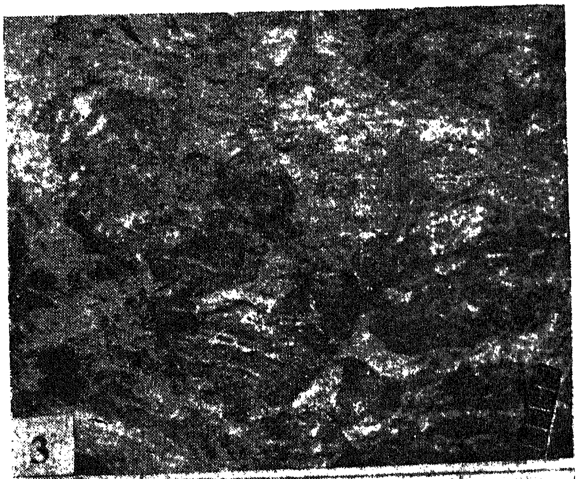


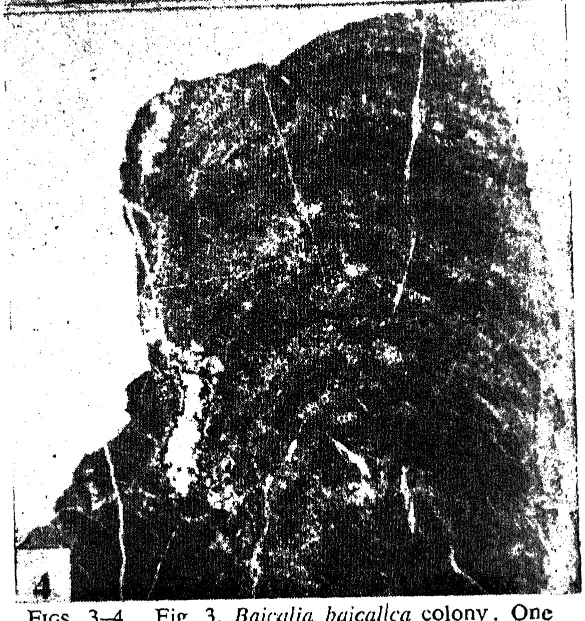
FIGS. 1–2. Fig. 1. Longitudinal section of *Conophyton garganicus*, showing well-defined axial zone, × 1. Fig. 2. Longitudinal section of *C. garganicus* showing arrangement of laminae, × 1.

Remarks.—The columns of *Baicalia baicalica* have not grown erect, but on a horizontal plane. Such growth of *Baicalia baicalica* is also present in Bhander limestone (Vindhyan) of Satna area (unpublished data), and is most probably due to hypersaline conditions. These stromatolites are phosphate bearing.

AGE OF KROL FORMATION

Age of Krol formation is highly disputed, mainly due to absence of any well-defined fossil assemblage. Most of the fossils and pollen and spores reported so far by various workers are of questionable stratigraphic value. Oldham[9] and most of the later workers assigned a presumed Permo-triassic age to Krol, because the underlying Blaini formation has been correlated with Talchir boulder bed, though without any fossil evidence.





FIGS. 3–4. Fig. 3. *Baicalia baicalica* colony. One div. of scale = 1 cm. Fig. 4. Section of *B. baicalica* showing internal structure. The laminae are fairly convex, and dark and light laminae possess gradational contact, × 1.

Sitholey *et al.*[10] and Lakhanpal[11] give Permo-triassic age to Infrakrol, while Ghosh and Srivastava[12], Sah *et al.*[13] give Triassic age to lower Krol. Tewari[14] assigns a Jurassic age, while Mittal and Chaturvedi[15] give a Permian to lower Cretaceous age; Sinha[16] also gives an upper Jurassic to lower Cretaceous age. However, Fuchs in Fuchs and Sinha[7] report that Infrakrol/Krol sediments yielded only primitive plant remains of no stratigraphic significance, and correlates Krol with Shali and assigns a Precambrian age. Gundu Rao[17] also reports Precambrian oncolites from Krol (identified by Mrs. Zhuravleva). Valdiya[18], based on palaeontological studies of Kalia[19], gave Permian age to Tal, and a lower Palaeozoic (Devonian) age to Blaini–Krol succession.

Thus, it is evident that no well-defined faunal and floral assemblage has been reported from the Krol sediments. In the present study a well-defined stromatolite assemblage of *Conophyton garganicus, Baicalia baicalica* and *Colonella* sp. has been recorded. This stromatolite assemblage has been considered a characteristic assemblage of Proterozoic (middle Riphean) of U.S.S.R. and Australia (Walter and Preiss[20]). In India this association has been reported from Gangolihat limestone and Vindhyan sediments, which are considered to be middle Riphean (Valdiya[21], Kumar[22], Kumar and Tewari[23]).

Singh and Tangri[24] reported *Conophyton* (?) and *Colonella* assemblage from Blaini formation and assigned a middle Riphean age. Singh[25] further suggested that Nagthat–Blaini–Krol–Tal succession is Precambrian in age, and lesser Himalaya is northern part of Indian shield. This find of Riphean stromatolites from Krol formation of Nainital further strengthens the contention of Singh[25].

CORRELATION

Conophyton has been reported from several Indian rocks, *e.g.*, Lower Vindhyan (Valdiya[21]; Kumar[22]); Bijawar (Krishna Murti[26]), Jammu limestone (Raha and Shastri[27]), Calc zone of Tejam (Bhattacharya[28]), Gangolihat dolomite (Kumar and Tewari[23]). However, except for Kumar[22], Kumar and Tewari[23] none of the workers made a study of internal laminae in thin section, which is essential in identification of stromatolites on generic and specific level.

Kumar and Tewari[23] report *Conophyton garganicus–Baicalia baicalica* assemblage from Gangolihat dolomite. Thus Krol formation can directly be correlated with Gangolihat dolomite of Calc zone of Pithoragarh. Nevertheless, the Krol formation can tentatively be put time equivalent and correlated to Calc zone of Pithoragarh, Shali limestone, Deoban limestone, Vindhyan sediments, and Aravalli phosphates, on the basis of presence of *Conophyton* sp. and *Baicalia* sp. in these rocks.

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1. Medlicott, H. B., *Mem. Geol. Surv. In* 1864, 3, 1.
2. Pilgrim, G. E. and West, W. D., *Ibid.*, 19 53, 1.
3. Auden, J. B., *Rec. Geol. Surv. India*, 19 67, 357.
4. Middlemiss, C. S., *Ibid.*, 1890, 23, 213.
5. Pal, D. and Merh, S. S., *Him. Geol.*, 19 4, 547.
6. Pande, I. C., *Ibid.*, 1974, 4, 532.
7. Fuchs, G. and Sinha, A. K., *Ibid.*, 1974, 563.
8. Davies, G. R., *Mem. Amer. Assoc. Pe Geol.*, 1970, 13, 169.
9. Oldham, R. D., *Rec. Geol. Surv. In* 1888, 21, 130.
10. Sitholey, R. V., Sah, S. C. D. and Du S. N., *J. Sci. Ind. Res.*, 1954, 13 B, 4
11. Lakhanpal, R. N., Sah, S. C. D. and Du S. N., *Palaeobotanist*, 1958, 7, 111.
12. Ghosh, A. K. and Srivastava, S. K., *Pr Nat. Inst. Sci. India*, 1962, 28 A, 710.
13. Sah, S. C. D., Venkatachala, B. S. a Lakhanpal, R. N., *Pub. Cent. Adv. Stu Geol., Punjab Univ.*, 1968, 5, 115.
14. Tewari, B. S., *Bull. Ind. Geol. Assoc.*, 196 2, 22.
15. Mittal, R. S. and Chaturvedi, R. S., *Ibid.* 1969, 2, 89.
16. Sinha, A. K., *Jour. Geol. Soc. India*, 197 16, 69.
17. Gundu Rao, C., *Publ. Centr. Adv. Stud. Geol.*, 1970, 7, 129.
18. Valdiya, K. S., *Jour. Geol. Soc. India*, 197 16, 119.
19. Kalia, P., *Proc. 2nd Ind. Colloq. Micro-palaeont. and Stratigraphy, Lucknow*, 1974, p. 107.
20. Walter, M. R. and Preiss, W. V., *Proc. 24th IGC*, 1972, p. 85.
21. Valdiya, K. S., *Jour. Geol. Soc. India*, 1969, 10, 1.
22. Kumar, S., *Jour. Palaeont. Soc. India*, 1976, 18, 13.
23. — and Tewari, V. C., *Jour. Geol. Soc. India*, 1977 (In press).
24. Singh, I. B. and Tangri, A. K., *Proc. 125th Ann. Celebr. Geol. Surv. India Symposium, Lucknow*, 1976.
25. —, *Ibid.*, 1976.
26. Krishna Murti, M., *Jour. Geol. Soc. India*, 1973, 13, 181.
27. Raha, P. K. and Shastri, M. V. A., *Him. Geol.*, 1973, 3, 135.
28. Bhattacharya, A. R., *Jour. Geol. Soc. India*, 1976, 17, 380.

UVEAL TISSUE AS AN ADJUVANT TO LENS PROTEIN

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ABSTRACT

The lens proteins are weakly antigenic and require the help of an adjuvant to produce antibodies. Bacterial proteins and toxins have been vairously cited as adjuvant *in vivo* although there is no convincing proof for this. The present work has demonstrated experimentally that whereas antibodies are not produced on injection of only lens or only uveal extract, they are produced when a mixture of lens and uveal extracts is injected into rabbits. The present work has also revealed that the uveal protein or pigment acts as an endogenous adjuvant in the producing antibody response.

INTRODUCTION

THE antigenic property of the lens was discovered in 1903[1] and it was later established that this antigenicity was responsible for the condition termed 'Endophthalmitis phacoanaphylactica'[2]. It is well established that the lens protiens are weakly antigenic and fail to produce any significant antibody response unless administered along with an adjuvant[3-8]. Burky[3] demonstrated the adjuvant action of staphylococcal toxins, and since then several workers have recorded that bacterial antigens and toxins act as 'adjuvant *in vivo*'[4-11].

In experimental animals also the necessity of using Freund's complete adjuvant[12-13] has been amply confirmed[11,14-17]. Muller's[7] comment on this issue sums up the consensus of opinion: "Low grade infection in humans which clinically might go unnoticed might possibly play the role of an adjuvant. Living or dead micro-organisms or toxins might act as adjuvant and in combination with liberated lens material lead to sensitisation and eventually to a phacogenic ophthalmia. Adjuvant may enter at the time of operation or by blood." It is evident that this explanation has been offered and accepted merely for want of a better one, since none of the workers was able to demonstrate a convincing source of bacterial toxins, whereas all workers merely presumed the presence of a hidden septic focus[3,7,11].

The present investigation was undertaken with a view to adducing experimental evidence to arrive at a more plausible explanation to this phenomenon. Further, during clinical work the present authors noticed that during extracapsular extractions there was often a considerable dispersal of uveal pigment while washing the anterior chamber of the eye and more so when pieces of capsule were pulled out with an Arruga's forceps. This led to the suspicion that the pigment or proteins from the uvea might be acting as an endogenous adjuvant to the lens protein to augment its effect.

EXPERIMENTAL

The experimental procedure was as follows:

1. Human cataractous lenses and goat uvea were used for the experiments. For each experiment three lenses were homogenised in 1·0 ml of normal saline and two uvea were homogenised in 1·0 ml of normal saline. The stock antigen solutions were prepared under completely sterile conditions and this was further confirmed by incubating the solutions at 37° C for 24 hours and inoculating the samples into blood agar plates. (Sodium azide 1/1000 was used as preservative.)

2. Four rabbits were immunised with neat lens antigen, four were immunised with neat uveal antigen and five rabbits were immunised with a mixture of equal volumes of lens and uveal antigen solution.

3. The schedule of immunisation was as follows: 0·5 ml of the antigen was injected subcutaneously at two different sites, once a week for one month. The sites of injection were changed each week. During the following month 1·0 ml of the antigen solution was injected intramuscularly into the thigh with an interval of two weeks between the two injections.

Trial bleeding was carried out one week after the last injection and the serum was obtained from each rabbit. This was stored at 20° C with sodium azide as preservative.

Ouchterlony's agar gel diffusion technique was employed to detect the presence of antibodies in the sera. A 1% agar solution (Difco Laboratories, U.S.A.) was prepared in barbitone buffer (pH 8·6) and allowed to set in thin layers on glass slides. Wells (3 mm diameter) were made in the gel in pairs 5 mm apart (Fig. 1). The paired wells were charged with the solutions as follows: In each pair one well (the left one in the figure) was charged with the serum while the other (the right one) was charged with either neat lens homogenate or neat uveal homogenate. The serum of all the experimental rabbits was thus tested against the lens and the uveal homogenate. The slides were kept at room temperature for 24 hours, humidity being maintained by keeping a wet filter paper in the petri-dish. The slides were then eluted in normal saline and kept in a refregirator for 24 hours. The uveal homogenate had consistently poor diffusion qualities in all the experiments.

RESULTS AND CONCLUSIONS

Figure 1 indicates the results of the experiments related to the reaction of antibodies with the lens homogenate. Examination of the figure leads to the following conclusions:

1. The serum from rabbits receiving neat lens antigen did not produce lines of precipitation thereby indicating that no antibodies were produced (Fig. 1-*a*).
2. Similarly the serum from the rabbits receiving neat uveal antigen also did not produce any line of precipitation indicating thereby that no antibody was produced in these too (Fig. 1-*b*).
3. The serum from all rabbits receiving the mixture of uveal and lens antigens produced one (Fig. 1-*d*) or two (Fig. 1-*c*) lines of precipitation when made to react with lens antigen. This indicates the presence of antibodies.

Reaction of antibodies with uveal homogenate was as follows:—

4. Sera of rabbits receiving neat lens and neat uveal antigen did not react with uveal antigen.
5. The serum of rabbits receiving the mixture of lens and uveal homogenate also did not show marked reaction with uveal antigen. Only a faint line of precipitation appeared in three of them.

From the above observations it is evident that individually the lens and the uveal protein are too weak to produce marked antibody response but when administered together they either potentiate each other's action or more likely the uvea augments the antigenicity of the lens protein. Probably the uvea acts as an adjuvant to lens protein as indicated by the fact that the antibodies formed in the serum after administration of the mixtue reacted better with lens protein than with uveal protein. The poor diffusion quality of the uveal homogenate does not, however, materially affect the conclusions drawn here.

Several workers[18–23] have noted a close association between sympathetic ophthalmitis (believed to be due to sensitisation to uveal pigment which is itself weakly antigenic[24–27]), and phacoanaphylactic uveitis. Blodi[22] found a 23% incidence (39/170) of phacoanaphylactic uveitis in eyes with clinically diagnosed sympathetic ophthalmitis. de Veer[18–19] suggested that phacoanaphylactic uveitis is occasionally the precursor of sympathetic ophthalmitis. He postulated that sensitisation to uveal tissue occurs following a destructive lesion produced by phacoanaphylactic uveitis. Eason and Zimmerman[23] stated—"···the almost constant occurrence of phacoanaphylactic uveitis in cases of sympathetic ophthalmitis was unanticipated and the observation requires explanation".

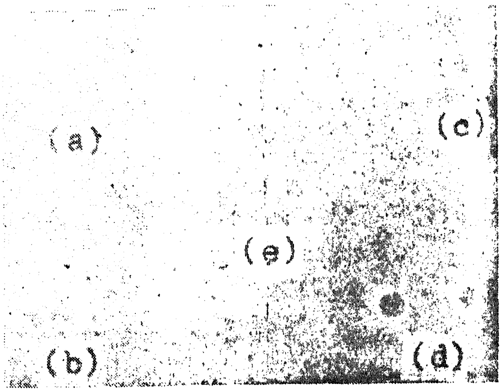

FIG. 1. Photograph of the agar gel plate 48 hours after charging the paired wells. The right well in each pair contained the lens homogenate. (*a*) The left well contained the antibodies from rabbits immunised with neat lens homogenate. (*b*) The left well contained the antibodies from rabbits immunised with neat uveal homogenate. No line of precipitation was noticed in the two cases (*a*) and (*b*). (*c*) and (*d*) The left well contained antibodies from rabbits immunised by a mixture of lens and uveal homogenate. Note the presence of one or two lines of precipitation due to antigen-antibody reaction. (*e*) Control in which the left well contained the antibodies from a rabbit immunised by a mixture of lens homogenate and Freund's complete adjuvant. Note the formation of the line of precipitation.

This explanation could well lie in the probability that the two antigens acted together, implying that the two different clinical diseases have a common basic etiopathogenesis, differing only in the nature of the response. One clinical picture dominates if the response is predominantly to one antigen whereas the other clinical picture dominates when the response is predominantly to the other antigen, although in both cases both the processes are simultaneously present. It is not unlikely that the 'perforating injury' of sympathetic ophthalmitis, which involves the iris and ciliary body, also damages the lens, at least to the extent that it may cause sufficient escape of lens proteins.

ACKNOWLEDGEMENT

We express our sincere thanks to Dr. B. S. Richur, M.D., Professor and Head of the Department of Pathology, Government Medical College, Nagpur, for his kind help.

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1. Uhlenhuth, P., 1903 (quoted by Francois, J., Rabaey, M., Wieme, R. J. and Kaminski, M., *Amer. J. Ophthal*, 1956, **42**, 577).

2. Verhoeff, F. H. and Lemoine, A. N., 1922 (quoted by Burky E. L., *Arch. Ophthal.*, 1934, **12**, 536).

3. Burky, E. L., *Arch Ophthal.*, 1934, **12**, 536.

4. Haik, G. M., Waugh, R. L., Jr., and Lyda, W., *Ibid.*, 1952, **47**, 437.

5. Scobes, R. G. and Slaughter, H. C., *Amer. J. Ophthal.*, 1956, **42**, 577.

6. Francois, J., Rabaey, M., Wime, R. J. and Kaminski, M., *Ibid.*, 1956, **42**, 577.

7. Muller H., *Trans. Ophthal. Soc. U.K.*, 1963, **83**, 687.

8. Lyda, W. and Lippincott, S. W., *Amer. J. Ophthal*, 1955, **40**, 120.

9. Brown, A., *Arch. Ophthal.*, 1934, **12**, 730.

10. Halbert, S P., Locatcher-Khorazo, D., Swick, L., Witmer, R., Segal, B. and Fitzgerald, P., *J. Expt. Med.*, 1957, **105**, 540.

11. Agrawal, L. P., Brar, B. S. and Goswamy, S., *Orient Arch. Ophthal.*, 1969, 7, 294.

12. Freund, J., *Ann. Rev. Microbiol.*, 1947, **1**, 291.

13. —, *Am. J. Clin. Path.*, 1951, **21**, 645.

14. White, R. G., Coons, A. H. and Connolly, J. M., *J. Expt. Med.*, 1955, **102**, 83.

15. Halbert, S. P. and Fitzgerald, P., *Amer. J. Ophthal.*, 1958, **46**, 187.

16. Luntz, M. H., *Exp. Eye Res.*, 1964, **3**, 166.

17. Selzer, G., Luntz M. H. and Sacks, M. I., *Amer. J. Ophthal.*, 1966, **61**, 1396.

18. de Veer, J. A., *Arch. Ophthal.*, 1953, **49**, 607.

19. —, *Ibid.*, 1940, **23**, 237.

20. Flocks, M., Litwinn, C. S. and Zimmerman, L. E., *Ibid.*, 1955, **54**, 37.

21. Kronenberg, A., *Amer. J. Ophthal.*, 1955, **40**, 205.

22. Blodi, F. C., *Trans. Amer. Acad. Ophthal.*, 1959, **63**, 642.

23. Easom, H. A. and Zimmerman, L. E., *Arch. Ophthal.*, 1964, 72, 9.

24. Luntz, M. H., *Amer. J. Ophthal.*, 1965, **59**, 373.

25. Aronson, S. B., Hogan, M. J. and Zweiger, P., *Arch. Ophthal.*, 1963, **69**, 105.

26. Collins, R. C., *Amer. J. Ophthal.*, 1949, **32**, 1687.

27. Suie, T. and Dodd, M. C., *Ibid.*, 1955, **39**, 377.

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LETTERS TO THE EDITOR

THE CRYSTAL STRUCTURE OF BIS HYDROGEN MALEATO TETRAAQUO IRON (II)

$Fe(C_4H_3O_4)_2 . 4H_2O$

THE title compound was studied to unravel the scheme of hydrogen bonds in the crystal as also to study the metal-oxygen ligands.

Crystal data: Triclinic with $a = 7{\cdot}31$, $b = 9{\cdot}69$, $c = 5{\cdot}24$ Å, $\alpha = 104{\cdot}7°$, $\beta = 90{\cdot}7°$, $\gamma = 114{\cdot}8°$, $Z = 1$, Space group $P\bar{1}$, $D_{obs} = 1{\cdot}83$, $D_{calc} = 1{\cdot}84$ gm/cc.

515 reflexions, zonal and three-dimensional, were collected using single cyrstal and Weissenberg photography with Cu K radiation. The intensities were estimated visually, data corrected for usual correction factors and brought to nearly absolute scale by statistical method.

Structure Determination, Refinement and Comments:

The structure has been solved in projections by the heavy-atom technique (heavy atom-occupying the special position 0, 0, 0 of centre of symmetry). Fourier syntheses gave the positions of the light atoms. The present R factors are: R (*hko*) = 0·12, R (*okl*) =0 ·14. A view of the structure looking down [001] is given in Fig. 1. In the structure, the metal is six-fold coordinated by oxygen atoms, metal–oxygen distances ranging from 1·96 to 2·21 Å. The dimensions of the maleate anion are normal. In the structure there are ten short distances ranging from 2·73 to 3·20 Å. Some of these may be real hydrogen bonds inclusive of bifurcated hydrogen bonds. A tentative scheme of hydrogen bonds is shown in the above figure. However, great interest lies in the location of the hydrogen bonds and in establishing the true scheme of hydrogen bonds in the structure. This part of the work is currently in progress.

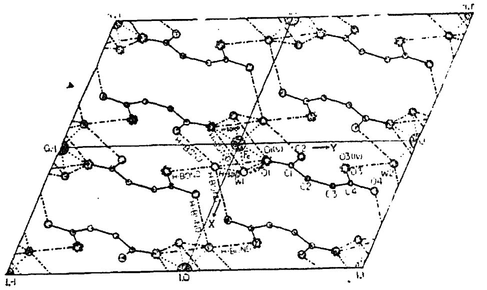

FIG. 1

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FERRIMAGNETIC VOLUME CHANGE IN LITHIUM-NICKEL FERRITES

THERMAL expansion of pure lithium ferrite has been studied by Haudek[1] and Naiden and Red'kin[2]. However, it is observed that no data on the thermal expansion of lithium-nickel ferrites are available in the literature. Also magnetic materials show interesting behaviour in the vicinity of the Curie temperature if the studies are extended to sufficiently high temperatures. With this aim, a study of the dependence of the coefficient of thermal expansion (α) of lithium-nickel ferrites on temperature and composition has been undertaken. The results thus obtained are presented in this communication.

Lithium-nickel ferrites of four different compositions having the chemical formula:

$$[(Li_{0\cdot5}\ Fe_{0\cdot5})_{1-\delta}\ Ni_{\delta}]\ Fe_2O_4$$

where δ ranges from 0 to 0·6, have been prepared starting from lithium carbonate (BDH 99% pure), nickel nitrate (BDH, 99% pure) and ferric oxide (99% pure) by the double sintering method. Rectangular bars are sintered between 1100° C and 1200° C depending on the composition in an oxygen atmosphere and are used in the present investigation.

The coefficient of thermal expansion for these ferrites has been determined over a temperature range 168° C to 725° C using the dilatometer technique[3]. Thermal expansion measurements are recorded at intervals of 50° C in general and 25° C to 30° C in the vicinity of the Curie temperature using a mechanical displacement dial gauge that could read to a thousandth of a mm. The accuracy in the determination of the value of α has been found to be ± 1%.

A plot of the coefficient of thermal expansion α *versus* temperature for the four ferrites is shown in Fig. 1. From the present study, it has been observed that the value of α increases gradually with the increase of temperature. All the four curves, however, show a hump in the vicinity of the Curie temperature, the maximum of the hump occurring exactly at the Curie temperature. This anomaly in the variation of the coefficient of thermal expansion near the Curie temperature can be attributed to the additional volume change caused by the destruction of the spontaneous magnetization. In the case of nickel and cobalt ferrites, Haudek[1] observed a similar variation of α with temperature with a hump in the vicinity of the Curie point. On the other hand, in the case of lithium ferrite he did not observe a similar hump, although the value of α showed an increase with increase of temperature. Naiden and Red'kin[2], however, have measured the coefficient of thermal expansion of lithium ferrite over a limited temperature range, *viz.*, 20° C–100° C. From the present investigation the value of α for lithium ferrite at 168° C is found to be $3{\cdot}1\times 10^{-6}$ per °C while the value reported by Naiden and Red'kin, over the temperature range 20° C to 100° C is about $6{\cdot}8\times 10^{-6}$ per °C, and that reported by Haudek[1] at 100° C is about $8{\cdot}0\times 10^{-6}$ per ° C. The low value obtained in the present investigation may be due to the high porosity (nearly 16%) and the difference in the conditions of preparation of the test specimens.

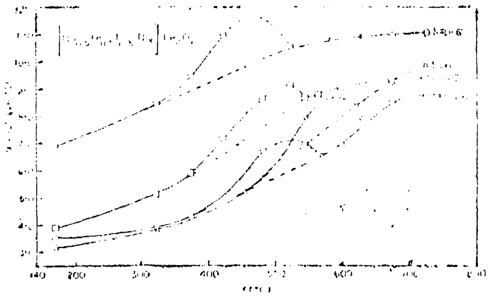

FIG. 1. Plot of coefficient of thermal expansion (α) as a function of temperature (in degree C) for lithium-nickel ferrites of different compositions.

From Fig. 1 it may be seen that for lithium ferrite, the peak occurs at 600° C, which lies in the range of values of Curie temperature reported in the literature[4]. The peak is observed to shift to the low temperature side as the nickel content is progressively increased showing thereby that the Curie temperature decreases with the increase of nickel content.

The ferrimagnetic volume change has been evaluated for the four ferrites by calculating the area between the hump and the base line indicated by dotted lines in the figure. The values of dV/V thus obtained are $0{\cdot}80\times 10^{-4}$; $0{\cdot}95\times 10^{-4}$; $1{\cdot}11\times 10^{-4}$ and $1{\cdot}88\times 10^{-4}$ for compositions with $\delta = 0$; 0·06, 0·3 and 0·6 respectively. It is also interesting to note that the value of dV/V increases with increasing nickel content.

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1. Haudek, H., *Z. Angrew Physik.*, 1971, **32**, 149.
2. Naiden, E. P. and Red'kin, G. A., *Izv. Vuz. Fiz.*, 1966, **2**, 168.
3. Ramana, Y. V. and Gogte, B. S., *Proceedings of Himalayan Geology Seminar* (Held in October 1976 at New Delhi).
4. *Hand Book of Microwave Ferrites*, Edited by Von Aulock, W. H., Academic Press, New York and London, 1965, p. 408.

FLAVONES AND FURANOFLAVONES FROM THE SEED SHELLS OF *PONGAMIA GLABRA*

Pongamia glabra is one of the commonest trees of India and has been extensively studied. The seeds[1-5], roots[6,7], stem bark, flowers[8], heart wood[9] and the leaves[10,11] have yielded a variety of flavones, chromenoflavones and furanoflavones.

The seed shells have not been examined so far. Recently, we have reported the components of the immature seeds[5] and now we report the chemical components of the seed shells. The immature seed shells (2·5 kg) were dried, powdered and extracted with benzene in a soxhlet extractor for 72 hrs. The benzene extract was concentrated and subjected to column chromatography over silica gel. Elution of the column with pet. ether yielded waxy materials (10·0 g) with low melting points. Further elution of the column with pet. ether-benzene (9 : 1) gave pongamol (100 mg, m.p. 128°) identified by comparison with an authentic sample. Pet. ether-benzene (7 : 3) eluate yielded karanjin (200 mg), m.p. 164°. Further elution of the column with pet. ether-benzene (1 : 1) furnished a yellow coloured oily liquid which was not studied. Elution of the column with pet. ether-benzene (1 : 2) gave first desmethoxykanugin (55 mg), m.p. 146-47° (lit.[7] m.p. 146°) followed by kanugin (60 mg), m.p. 216–17° (lit.[7] m.p. 204°). Elution of the column with pure benzene yielded pongapin (200 mg), m.p. 191° (lit.[1] m.p. 191°). Further elution with benzene-ethyl acetate (98 : 2) furnished kanjone (150 mg), m.p. 190–91° (lit.[1] m.p. 191–92°). Final elution of the column with benzene-ethyl acetate (9 : 1) gave pinnatin (100 mg), m.p. 184-85° (lit.[12] m.p. 183–84°).

Thus the immature seed shells contain pongamol, karanjin, desmethoxykanugin, kanugin, pongapin, kanjone and pinnatin while immature seeds[5] have karanjin, pongapin, lanceolatin-B, kanjone and isopangaflavone. Thus we find that all the components present in the seeds and seed shells have resorcinol unit except pinnatin. The shells have small amounts of desmethoxykanugin and kanugin which are the main components of the stem bark[6,7]. Thus the seed shells have components some of which are present in the stem bark while others are present in the seeds.

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1. Aneja, R., Khanna, R. N. and Seshadri, T. R., *J. Chem. Soc.*, 1963, p. 163.
2. Khanna, R. N. and Seshadri, T. R., *Tetrahedron*, 1963, **19**, 219.
3. Naik Satam, P. G. and Bringi, N.V., *Indian J., Chem.*, 1973, **11**, 1188.
4. — and —, *Ibid.*, 1973, **11**, 209.
5. Roy, D., Sharma, N. N. and Khanna, R. N., *Ibid.* (In press).
6. Mukerjee, S. K., Sarkar, S. C. and Seshadri, T. R., *Ibid.*, 1969, **7**, 1275.
7. —, — and —, *Tetrahedron*, 1969, **25**, 1063.
8. Murti, P. B. R. and Seshadri, T. R., *Proc. Indian Acad. Sci.*, 1944, **28**, 279.
9. Subrahmanyam, K., Rao, J. M. and Rao, K. V. J., *Indian J. Chem.*, 1977, **15 B**, 12.
10. Malik, S. B., Seshadri, T. R. and Sharma, P., *Ibid.*, 1976, **14 B**, 229.
11. Khanna, R. N. and Seshadri, T. R., *Ibid.*, 1963, **1**, 385.
12. Pavanaram, S. K. and Row, L. R., *Aust. J. Chem.*, 1956, **9**, 132.

EOSIN AS A RE-CONCENTRATOR IN THE DETERMINATION OF ANTIMONY IN NATURAL WATERS BY NEUTRON ACTIVATION

Introduction

As many elements exist in natural waters at trace concentrations and they are admixed with major constituents like sodium, potassium, magnesium, calcium and chloride which interfere in the direct determination, many workers have attempted to absorb them on various precipitates such as manganese dioxide, ferric hydroxide[1], phosphates[2], organic precipitants, organic[3] and inorganic ion exchangers. To provide a sensitive technique, neutron activation method has been applied to determine antimony in seawater in concentration of parts per billion or less, using the co-precipitation of anitmony on eosin in acidic medium. The theoretical sensitivity by neutron activation for antimony is reported[4] as 4×10^{-5} μgm based on 2·8 d ^{122}Sb and 3×10^{-4} μgm based on 60 d ^{124}Sb for an induced activity of 40 dps for a thermal neutron flux of 5×10^{13} n/cm^2/sec.

Analytical Procedure

One litre of filtered seawater was acidified to 1·5 N with concentrated HCl. Eosin (300 mg) was added to this, stirred and kept overnight and then filtered through millipore filter paper (0·45 μ size). The precipitate was dried, packed in an aluminium foil, sealed in an aluminium can and irradiated under a neutron flux of about 10^{13} n/cm^2/sec for 24 hours. The sample was cooled for 3 days and then taken for further chemical analysis.

The irradiated samples were dissolved in conc. HNO_3, 30 mg Sb carrier (Sb Cl_3 solution) added and further ashed by HNO_3 till the colourless residue was obtained. The residue was taken up in 15 ml of 6 N HCl. The solution was passed through Dowex-1 × 8 (50–100 mesh, 6 mm × 10 cm) column pre-equilibrated, with 6 N HCl. The column was washed gradually with 5 N HCl, 15 ml of N HCl and finally with 10 ml of 9 M H_2SO_4. Sb was eluted after washing the column with 2 ml of H_2O followed by 15 ml of boiling 2 M NaOH[5]. The eluates were evaporated to dryness, adjusted to 3–4 N HCl and reduced to metallic antimony by the addition of iron powder[6]. The metal was filtered on millipore filter paper, washed twice with 0·5 N HCl, dried under 110 °C and assayed for ^{124}Sb, using well type NaI (Tl) crystal coupled to a 512 Channel Analyser.

Aliquots of standard solution, varying between 0·1–0·2 μg, were carried out on 300 mg eosin under identical conditions and irradiated along with the sample in the reactor. The radiochemical processing of the standard was done essentially in the same way as that of sample described above. Quantitative determinations of the samples were done by comparing with this standard.

Results and Discussion

Preliminary experiments were carried out to study the specificity of eosin for Sb in presence of other nuclides using 200 ml of filtered seawater, acidified to 0·1 N with conc. HCl, spiked with known amounts of ^{125}Sb tracer and other tracers such as ^{95}Zr, ^{133}Ba, ^{106}Ru, ^{65}Zn, ^{85}Sr, ^{60}Co and ^{54}Mn. Eosin (200 mg) was added to this, stirred for 2 minutes, allowed to settle overnight and then filtered through millipore filter paper (0·45 μ size). The filter paper was transferred into counting vial and eosin dissolved in minimum volume of alcohol. The height of the liquid was adjusted to the same as that of the standard and counted. It was observed that ^{125}Sb was carried to significant level (65%) followed by ^{75}Se (18·85%) and ^{95}Zr (17·04%). Isotopes of Ba, Ru, Zn and Sr were carried insignificantly (less than 2%) while Co and Mn were not carried even to the detection levels.

Experiments were conducted to arrive at the optimum acidity condition for the co-precipitation of Sb on eosin. The acidity of samples was changed from 0·1 N to 4·0 N HCl. Results given in Fig. 1 show that eosin carried ^{125}Sb to the extent of 94% at 1·5 N and further increase of acidity did not improve the yield significantly. Study was made to ascertain the extent of decontamination of ^{95}Zr and ^{75}Se at the optimum acidity condition of 1·5 N. The carrying ^{75}Se on eosin was decreased considerably to 3·24% at this acidity compared to 18·35% at 0·1 N HCl; ^{65}Zn was not carried on it but the contribution due to ^{95}Zr remained almost the same (16·98%) as that at 0·1 N HCl. However, the contamination of ^{95}Zr is eliminated by the radiochemical procedure further followed as described above.

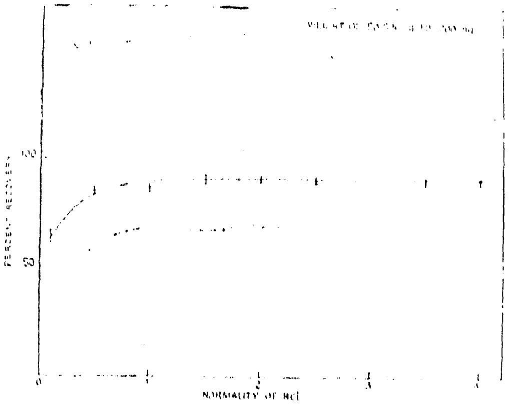

FIG. 1. Recovery of ^{125}Sb on eosin at different normalities of Hcl,

Attempt was made to find the minimum amount of eosin needed to carry Sb quantitatively from one litre of seawater; 200–400 mg eosin were added to spiked seawater of 1·5 N acidity. Recoveries obtained for ^{125}Sb on 200 mg and 300 mg of eosin were 94·81 ± 1·54% and 98·73 ± 1·58% respectively. It is observed that 200 mg of eosin is sufficient to carry ^{125}Sb almost quantitatively from 1 litre of seawater. As the sensitivity of detection by neutron activation for nuclear reaction ^{123}Sb (n, γ) ^{124}Sb is quite high (3×10^{-4} μg Sb), 500 ml to 1 litre of seawater is enough for its determination.

It was attempted to study the possible variation in the carrying efficiency of 200 mg of eosin at different concentrations of stable antimony carrier by spiking 1 litre seawater with antimony in the range 0·5–10 μgm along with ^{125}Sb tracer. The results given in Table I indicate that the carrying yield has remained substantially at the same quantitative level throughout the range of Sb concentration studied.

TABLE I

Effect of antimony carrier on ^{125}Sb recovery from 1 litre of seawater

Expt. No.	Antimony carrier added (μm)	No. of counts added counts/min	No. of counts recovered counts/min	Recovery %
1	0·5	747	723	96·79 ± 1·61
2	1·0	779	756	97·16 ± 1·56
3	2·0	779	768	98·60 ± 1·59
4	4·0	779	768	98·60 ± 1·59
5	5·0	747	751	100·60 ± 1·63
6	10·0	747	737	98·68 ± 1·62

If ^{233}U or ^{235}U is present in the sample, a number of radioactive isotopes of Sb will be produced by fission. This will lead to the increase in the production of ^{124}Sb produced by thermal neutron activation. In order to confirm whether uranium is carried on eosin, 300 mg of eosin were added to 1 litre of seawater, spiked with 500 μgm uranium and adjusted to 1·5 N HCl. The eosin precipitate after overnight settling was filtered, were digested with conc. HNO_3 and then counted for alpha activity. The absence of any alpha activity confirmed that the carrying capacity of eosin for uranium is negligible.

Determination of antimony in aquatic samples from different locations was carried out and the results are given in Table II. The concentration range of Sb in seawater by neutron activation analysis is comparable with those obtained by other workers[7,8].

TABLE II

Antimony content of surface water samples

Location	μg/litre × 10^{-3}
Fresh water collected from Thana Tank	3·5 ± 0·003
Seawater from Cirus Jetty	9·6 ± 0·012
Seawater from Kalwa Bridge	14·0 ± 0·015

The entire radiochemical procedure after irradiation takes not more than 4 hours and hence it is advantageous in carrying out antimony estimations in large number of samples. As the volumes of seawater required by this method are very small as compared to those required for conventional chemical method, this technique will be useful to study the distribution of Sb in seawater.

Thanks are due to Dr. A. K. Ganguly, Director, Chemical Group, and Shri S. D. Soman, Head, Health Physics Division, Bhabha Atomic Research Centre, for their interest during the course of this work.

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---

1. Doshi, G. R., Krishnamoorthy, T. M., Sastry, V. N. and Sarma, T. P., *Indian J. Chem.*, 1973, **11**, 158.
2. Doshi, G. R., *Ibid.*, 1967, **5**, 580.
3. Davies, R. V., Kennedy, J., Packett, J. W. A., Robinson, B. K. and Streetson, R. J. W., *United Kingdom AEC Report No. AERE-R*–5024, 1965.
4. Leddicotte, G. W. and Reynolds, S. A., *Nucleonics*, 1951, 8 (3), 62.
5. Manfred Linder, *United States AEC Report–UCRL*–14258, Livermore, California, 1965.
6. William, J. Maeck, *The Radiochemistry of Antimony*, National Academy of Sciences—National Research Council, 1961.
7. Stanely, E. Thompson, Ann Burton, C., Dorothy, J. Quinn, and Yook, C. Ng., *United States AEC Report*, 50564, 1972, Rev. 1.
8. Portman, J. E. and Riley, J. P., *Analytica Chimica Acta*, 1966, **35** (1), 35.

INELASTIC SCATTERING OF 280 keV PHOTONS BY K-SHELL ELECTRONS OF LEAD, TUNGSTEN AND TIN

INELASTIC scattering of a photon by an electron is described by Klein-Nishina formula where it is assumed that the struck electron is initially free and at rest. However, the effect of electron binding has to be considered for low energy photons and for inner shell electrons where the incident photon energy is comparable with the binding energy of the electron. There have been some investigations[1-5] of inelastic scattering of gamma rays by K-shell electrons, but most of these measurements are confined to high energy photons. In order to obtain data at lower energies we have measured the differential K-shell Compton scattering cross-sections of 280 keV photons from lead, tungsten and tin at a scattering angle of 90°.

When a photon is Compton scattered by an electron bound in the K-shell of the atom, the electron is ejected from its orbit and a K X-ray is emitted with a certain probability defined by the K-shell floursecent yield. The experiment consists of measuring the intensity of scattered photons in coincidence with the K X-rays, thus eliminating the scattering taking place from electrons of other shells. The beam of 280 keV photons is obtained by collimating the radiations from 2 curie ^{203}Hg source. Gamma rays and X-rays are detected with 51 mm and 2 mm thick NaI (Tl) scintillation detectors respectively. The X-ray detector is placed at 90° to the scattering plane to minimize detector to detector scattering. The resolving time of the coincidence set-up is of the order of 35 n sec. The measurements are made for the elements of lead, tungsten and tin at a scattering angle of 90°. The measured coincidence counts are corrected for (i) chance coincidence, (ii) multiple scattering, (iii) bremmstrahlung radiation and K-shell X-rays produced by photo and Compton electrons, (iv) cosmic ray background and (v) photons scattered from one detector to other detector.

The results obtained are in the form of ratio of differential scattering cross-section of K-shell electron ($d\sigma_k$) to that of free electron ($d\sigma_k$) and are shown in the column III of Table I. Theoretical results[6] calculated on the basis of non-relativistic wave function of K-shell electron in the hydrogen like atom are shown in column IV. While for lead and tungsten there is agreement with theory within experimental error, for tin the measured cross-section is rather large.

TABLE I

Differential cross-section ratio $d\sigma_k/d\sigma_f$ *for 280 keV photons at* 90°

Element	Z	$(d\sigma_k/d\sigma_f)_{exp.}$	$(d\sigma_k/d_f)_{th.}$
Lead	82	0·308 ±0·045	0·300
Tungsten	74	0·401 ±0·047	0·366
Tin	50	1·037 ±0·051	0·803

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Patiala, India, *September* 19, 1977.

1. Motz, J. W. and Missoni, G., *Phys. Rev.*, 1961, **124**, 1458.
2. Varma, J. and Eswaran, M. A., *Ibid.*, 1962, **127**, 1197.
3. Ramalinga Reddy, A., Lakshminarayana, V. and Jananananda, S., *Proc. Phys. Soc.*, 1967, **91**, 71.
4. Pingot, O., *Nucl. Phys.*, 1968, **A119**, 667.
5. —, *Ibid.*, 1969, **A133**, 334.
6. Shimizu, S., Nakayaâa, Y. and Mukoyama, T., *Phys. Rev.*, 1965, **140**, A806.

SOME HISTOPATHOLOGICAL AND HISTOCHEMICAL OBSERVATIONS IN THE TESTICULAR TISSUES OF VASECTOMIZED AND CAUDECTOMIZED RAMS*

BILATERAL vasectomy and caudectomy are dependable surgical methods for making teaser rams which are extensively employed in sheep husbandry. Basic physiopathological changes have been investigated histochemically in dog[1] and man[2] in order to evaluate the long term effects.

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* Part of research work embodied in the M.V.Sc. Thesis, Agra University, Agra, 1975, by K. Tandon.

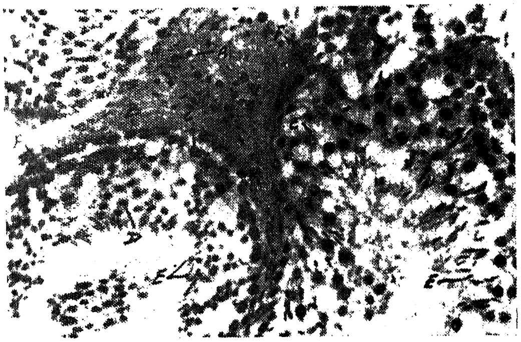
Post-surgical changes consequent to vasectomy and idectomy in ram were examined histopathologically l histochemically in the present investigation.

aterial and Methods

Bilateral vasectomy and caudectomy were performed a group of five rams each and the testicles were gically removed from each animal 4–6 months post-ratively. During this interval other physiological ameters pertinent to the work were studied[3]. Testi- ar tissue intended for alkaline phosphatase activity e fixed in chilled acetone and stored under refrige-on. The tissues required for histopathological ervations were fixed in neutral formalin. H and E l trichome staining were employed in the histo-hological investigation. The polysaccharides were lonstrated with PAS reaction. Alkaline phos-tase was demonstrated by Gomoris-method as mmended by Davenport[1]. All these staining tech-ies are described in detail by Lillie[5].

ults and Discussion

xamination of the testes of vasectomised rams aled spermatogenesis in the majority of semini-us tubules. These tubules were lined by Sertoli s and various stages of spermatogenic cells (Fig. 1).



G. 1. Section of testis six months post-vasectomy ram. H and E (× 280). A. Highly vacuolated of Leydig. B. Spermatogonia. C. Primary matocyte. D. Spermatids. E. Nucleus of Sertoli

trary to these findings it was mentioned that vasec-y caused back pressure in the seminiferous tubules induced degeneration of germinal epithelium[6]. interstitial tissue in vasectomized rams frequently led conspicuous cells of Leydig with extensive olation in the cytoplasm (Fig. 1). In bulls such inge was attributed to the androgenic content of testis[7]. In man, such a change could lead to re-ration of ageing males by improving endocrine ions of the testis[6]. It is also speculated that ased quantity of androgens is secreted in this p of animals. In caudectomized group, large areas of the testis revealed complete degeneration of seminiferous tubules and the presence of strands of collagenous fibres along with extensive cellular infiltration (Fig. 2). The interstitial tissue revealed extensive cellular infiltration, fibroblastic predominance with cells of Leydig, hardly distinguishable.

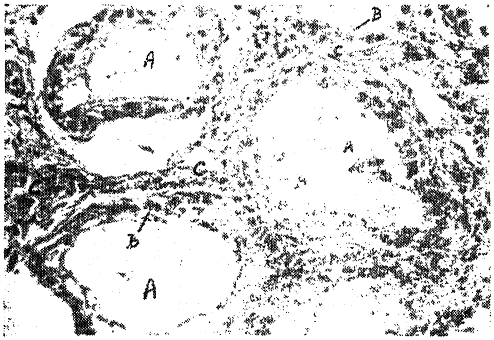


FIG. 2. Degeneration of seminiferous tubules (A), the germinal epithelium (B) and the interstitial tissue (C): section of testis three months post-caudectomy from a ram. H and E (× 280).

Histochemical examination of the testis in vasectomized rams showed mild to moderate alkaline phosphatase activity in the spermatogonia, spermatocytes, spermatids and Sertoli cells. Activity was intense in the heads of spermatozoa. In caudectomized specimen the lining cells of the seminiferous tubules showed negative to mild alkaline phosphatase activity.

With PAS the basement membrane of the seminiferous tubules in the testis of rams of both group appeared mildly positive. The cytoplasm of various germinal cells and Sertoli cells was, however, mildly PAS positive and contained moderate amounts of acid mucopolysaccharides. Glycogen was, however, not demonstrable in both the groups of rams in the present investigation. Alkaline phosphatase has actually been considered an excellent marker of the germ cells in the developing testis[8], its progressive decline in the nuclei is a characteristic feature of the spermatogenetic process.

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1. Kothari, L. K. and Mishra, P., *Int. J. Fert.*, 1973, **18**, 119.
2. Gupta, A. S., Kothari, L. K., Khruva, A. and Bapna, R., *Br. J. Surg.*, 1975, **62**, 59.
3. Tandon, K., *M.V.Sc. Thesis*, Surgery, Agra University, Agra, 1974.
4. Davenport, H. A., *Histological and Histochemical Technics*, Saunder Co., Philadelphia, 1960.
5. Lillie, R. D. *Histopathologic Technic and Practical Histochemistry*, McGraw-Hill, New York, 1965.
6. Steinach, E., *Med. J. Rec.*, 1927, **125**, 77.
7. Hooker, C. W., *Am. J. Anat.*, 1944, **74**, 1.
8. Johnson, A. D., Gomes, W. R. and Vandemark, N. L., *The Testis*, Academic Press, New York, 1970, **2**.

STUDIES ON THE PEARL OYSTER, *PINCTADA FUCATA* (GOULD) OF THE GULF OF KUTCH

III. Preliminary Investigation on Pearl Culture

INDIAN pearl fishery is restricted to two regions namely the Gulf of Mannar in Southern India and the Gulf of Kutch (Jamnagar district) in Gujarat. The pearl fishery of Gulf of Kutch is comparatively small and is at standstill for the past few years. Unlike the pearl oysters of the Gulf of Mannar, the pearl oyster, *Pinctada fucata* (Gould) of the Gulf of Kutch is intertidal in habitat and could easily be picked up. Possibly because of this, the pearl fishery at Jamnagar coast flourished some years back. In spite of the existence of potential grounds of the pearl oysters, so far, no experiments centering around pearl culture have been undertaken. With a view to investigating the potentiality for pearl formation, various indigenously prepared nuclei in comparison with imported Japanese nuclei were employed in the pearl oyster, *P. fucata*.

One hundred and eighty-five live *P. fucata* (3 to 5 years) were collected from Piroton, Ajad, Deeda and Moovada islands of the Gulf of Kutch during October-November 1974. The pearl oysters were maintained in protected marine impoundments at Sikka coast, Jamnagar, Gujarat, by keeping them in Japanese designed rafts (under 3′ of water). No mortality was reported.

During January 1975, the oysters were taken out in groups of 10 to 15 from the rafts and after washing, were exposed to bright sunlight. when the oysters opened the valves within five minutes, a piece of wood (4″ long and 1/2″ wide with tapering) was inserted inside each oyster kept in trays filled with sea water. Nucleus of each type was inserted in the gonadial region of each oyster over which mantlepiece (1 cm square size) was kept. Precautions were taken to see that the soft body parts of the oysters were not injured. Oysters were then carefully transferred back to the raft. Each oyster was kept separately in a petridish (9″ dia.) The types of nuclei (2-3 mm dia.) used in the present study are given in Table I.

TABLE I

Results of pearl culture

Types of the nuclei inserted	% of Rejection	% of Retention	% of Mortality
1. True pearl	40	60	44
2. Pearl from *Placuna placenta*	60	40	30
3. Imported Japanese Nuclei	80	20	36
4. Toothpastefoil	80	20	00
5. 'Mala moti'	20	80	00
6. Partially coated pearls	100	00	20

Long sterilised instruments were used. About 50 pearl oysters of age group 3 to 5 years were used for seeking results for each type of the nuclei as mentioned in Table I. The nuclei of all types inserted were ejected within a week's time after the operations (Table I). After this period none of the pearl oysters ejected the nuclei. This indicated that the operated pearl oysters should be kept under observation for a week (rejection period) after insertion of nuclei.

Algarswamy and Quasim$^{1-3}$ maintained the pearl oyster, *P. fucata* of the Gulf of Mannar in standing sea water in the laboratory before, and, after operations and narcotised the pearl oysters before the operations. They also reported that the operated pearl oysters ejected the implanted nuclei (4 hours to 2–3 days after operation).

The pearl oysters implanted with white holed nuclei (Used in Mala) and with particles prepared from foil suffered no mortality (Table I) but rejection of the foil nuclei was 80% ; in the case of the former, the rejection was 20% and the retention of the nuclei resulted in beautiful lustrous pearls with bluish tint (80%). Among other types of the nuclei tried, true pearls (pearls of small size) deserve attention. In this case the retention was 60%, rejection 40%, and mortality 44%. Mortality seems to be a factor of serious concern here. Similarly partially coated nuclei were also rejected by the pearl oysters and mortality was 20%. Imported Japanese nuclei caused 36% mortality, and 80% rejection. From these studies it can be stated that imported nuclei from

Japan are not suitable for pearl culture in *P. fucata* of the Gulf of Kutch. Further work is in progress.

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---

1. Algarswami, K., *Proc. Symp. Mollusca III*, Mar. Biol. Ass. India, 1970, p. 975.
2. — and Quasim, S. Z., *Indian J. Fish.* 1970, 20, 553.
3. — and —, *Sea Food Expt. J.*, 1970, 6, 1.

MICROSPOROGENESIS IN *GILIA CAPITATA* SIMS

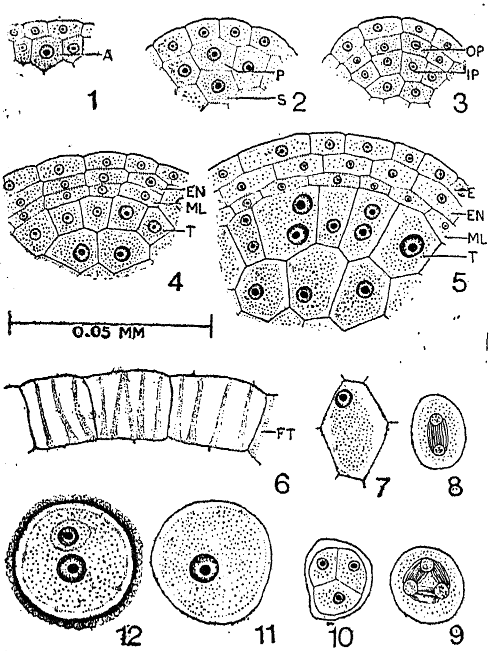
THE embryology of Polemoniaceae has received very little attention from earlier workers[1]. Souèges[2-4] described the embryogeny in *Polemonium caeruleum*, while Sundar Rao[5] worked out the development of its male and female gametophytes. This note reports on the microsporogenesis in *Gilia capitata*.

A mature anther consists of an outer layer of epidermis, single layer each of endothecium and middle layer and an innermost tapetum (Fig. 5). The cells of the epidermis are cuticularized while those of the endothecium develops characteristic fibrous thickenings (Fig. 6). The cells of the middle layer are rectangular and are ephemeral and the tapetal cells are two-nucleate which finally become one-nucleate by the fusion of nuclei.

A young anther is a homogeneous mass of parenchymatous tissue. During further development, it becomes four lobed and in each lobe a single hypodermal archesporial cell is differentiated. This is distinguishable from other cells by larger size, dense cytoplasm and conspicuous nucleus (Fig. 1). The first periclinal division of the archesporium results into an outer primary parietal cell and an inner primary sporogenous cell (Fig. 2). The outer primary parietal cell divides periclinally to form a secondary outer and a secondary inner parietal layer (Fig. 3). The outer secondary parietal layer gives rise to two layers by a periclinal division, the outer forms the endothecium and the inner the single middle layer (Fig. 4). The inner secondary parietal layer develops directly into the tapetum. The development of the anther wall conforms to the dicotyledonous type[1].

The sporogenous cell divides to form a few cells and the last division forms the microspore mother cells. Each microspore mother cell undergoes the usual reduction division simultaneously to form a tetrahedral tetrad (Figs. 7–10).

The microspores are nearly triangular at the time of separation from the tetrad and at maturity become almost round (Fig. 11). A young microspore is rich in cytoplasmic contents and has a large nucleus. Soon it enlarges considerably and the exine develops ornamentations (Fig. 12). Its nucleus divides to give rise to a vegetative and a generative nucleus. The pollen grains are shed at bicelled stage (Fig. 12).



FIGS. 1–12. Microsporogenesis in *Gilia capitata*. Figs. 1–5. Development of anther wall layers and stages in microsporogenesis. Fig. 6. Cells of the endothecium showing fibrous thickenings. Figs. 7–10. Stages in the development of the microspore. Fig. 11. A microspore. Fig. 12. Two celled pollen grain. (A—Archesporial cell; E—Epidermis; EN—Endothecium; FT—Fibrous thickenings; IP—Inner secondary parietal layer; ML—Middle layer; OP—Outer secondary parietal layer; P—Primary parietal layer; S—Primary sporogenous layer; T—Tapetum.)

During maturation of the anther, the epidermal cells shrivel and the cells of the middle layer disintegrate. The anther wall at the time of dehiscence comprises of a degenerated epidermis and an endothecial layer. The dehiscence is by a longitudinal slit.

The authors are grateful to Dr. V. Singh for laboratory facilities and for going through the manuscript.

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1. Davis, G. L., *Systematic Embryology of Angiosperms*, John Wiley and Sons, Inc., New York, 1966.
2. Souèges, R., *C.R. Acad. Sci. Paris*, 1939, **208**, 1338.
3. —, *Bull. Soc. Bot. France*, 1942, **86**, 289.
4. —, *C.R. Acad. Sci. Paris*, 1945, **220**, 837.
5. Sundar Rao, Y., *Proc. Natl. Inst. Sci. India*, 1940, **6**, 695.

A NEW RECORD OF *ALTERNARIA* CAUSING LEAF SPOT OF SUNFLOWER

A SEVERE form of leaf spot due to *Alternaria* was observed during kharif 1973 on sunflower (*Helianthus annus* L.) crop grown around Srikalahasti in Chittoor district of Andhra Pradesh. The disease develops initially as small, chlorotic spots with brown centre on young leaves (Fig. 1 *a*). These spots turn dark brown and are somewhat circular with yellow halos measuring about 3–5 mm in diameter after 10 days (Fig. 1 *b*). They later coalesce forming irregular dark patches ultimately leading to severe blighting, drying up of leaves and defoliation by 20 days (Fig. 1 *c*).

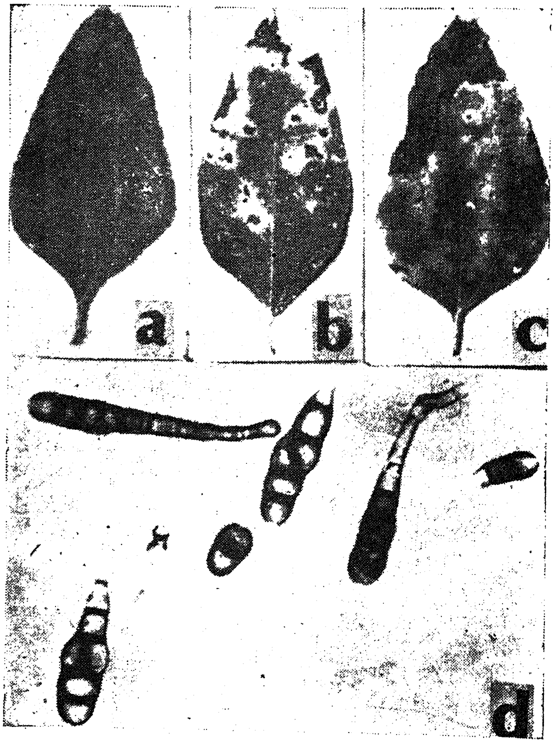

FIG. 1. *a*, *b* and *c*: Progression of symptoms on the leaves of sunflower due to *Alternaria helianthicola* (Rao and Raj), causing leaf spot; *d*: Conidia of *A. helianthicola* showing tapered apex, beak and longitudinal septa in some.

The pathogen was purified by single spore isolation and the pathogenicity of the organism was proved with positive results in sunflower var. E.C. 68414, E.C. 68415 and Bulgarian. The organism failed to infect ten known hosts of *Alternaria*, viz., *Cyamopsis tetragonoloba* (L.) Taub., *Raphanus sativus* L., *Glycine max* (L.) Merrill, *Arachis hypogaea* L., *Solanum melongena* L., *Lycopersicon esculentum* Mill., *Chrysanthemum maximum* L., *Zinnia elegans* Jacq., *Cucumis sativus* L. and *Brassica juncea* Coss. and appeared to be host specific.

Mycelial colonies on potato dextrose agar were dark, profusely branched and frequently septate with abundant sporulation in 7–10 days. The conidiophores were cylindrical, often branched, septate and difficult to be ditinguished from the mycelium. Conidia were golden yellow or dark brown, ellipsoidal, each having tapered apex and distinct beak, 2–10 septate, slightly constricted at septation. The conidia inclusive of beak measured 66·5 (29·0–91·5) × 15·6 (7·25–21·75) μ, beak alone measuring 45·5 (29·0–72·5) μ in length. Longitudinal septae were present in some conidia (Fig. 1 *d*). Characters of conidia produced in culture were similar to those observed in nature.

From the perusal of literature, it is seen that *A. tenuis*[1], *A. zinniae*[2] and *A. helianthi*[3,4] infect sunflower and cause different leaf spots. However, the foliar symptoms, host range, sporulation as well as conidial measurements of the present isolate differ wholly or partly from the above species of *Alternaria* reported on sunflower. The isolate has since been examined and found quite distinct from *A. tenuis*, *A. zinniae* and *A. helianthi* and hence named as *Alternaria helianthicola* sp. (Rao and Raj.) now, as suggested by Dr. Ellis of Commonwealth Mycological Institute, Kew, Surrey, England. The type culture has been deposited at C.M.I., Kew, Surrey, England, as IMI 191573.

A Latin description of the new species is given below: "Mycelium (in cultura) fuscum, ramosissimum et saepe septatum, sporulatione copiosa; conidiophora cylindrica, saepe ramosa, septata, a mycelio difficile distinguibilia; conidia aurea vel atrobrunnea, ellipsoidea, unumquidque apice contracta et rostro distincto, 2–10 septatum, ad septam leviter constrictum; conidia (rostro incluso) 66·5 (29·0–91·5) × 15·6 (7·25–21·75) μ; rostrum tantum 45·5 (29·0–72·5) μ longum; septa longitudinalia conidiis nonnullis praesentia".

Our grateful thanks are due to Dr. Ellis and Mr. Anthony Johnston, Director, C.M.I., England, for examining the culture and suggesting the new name. Thanks are also due to Rev. Father K. M. Matthew, S.J., Director, The Rapinat Herbarium, St. Joseph's

College, Tiruchirapalli, Tamil Nadu, for Latin description of the new species.

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---

1. Bose, A. B., *J. Ind. Bot. Soc.*, 1943, **21**, 179.
2. McDonald, W. C. and Martens, J. W., *Phytopathology*, 1963, **53**, 93.
3. Tubaki, K. and Nishihara, N., *Trans. Brit. Mycol. Soc.*, 1969, **53**, 147.
4. Anilkumar, T. B., Shantha, D. Urs, Seshadri, V. S. and Hegde, R. K., *Curr. Sci.*, 1974, **43**, 94.

AN IMPROVED TECHNIQUE FOR THE KARYOTYPE STUDY OF AN ECONOMICALLY IMPORTANT FAMILY UMBELLIFERAE

INDIA has got a large number of Umbelliferous species many of which are economically important as spices, vegetables and medicinal plants. Chromosome number of Umbellifers has been studied by many authors (*Wanscher, 1933; *Delay, 1947; *Garde and Garde, 1949, 1954; *Hakansson, 1953; *Sharma and Ghosh 1954; *Sharma and Bhattacharyya, 1959; *Bell and Constance, 1960, 1966; *Gadella and Kliphuis, 1967; *Cauwet, 1971). With one or two exceptions detailed studies of chromosome morphology of the members of this family which can elucidate structure and behaviour of karyotype and their interrelationships are yet to be made. Moore[3] pointed out that Umbelliferous cytotaxonomy is still at the 'alpha' level. This work involved the evolution of special pretreatment and fixation schedules for a proper study of chromosome morphology of members of Umbelliferae and their cytological implications.

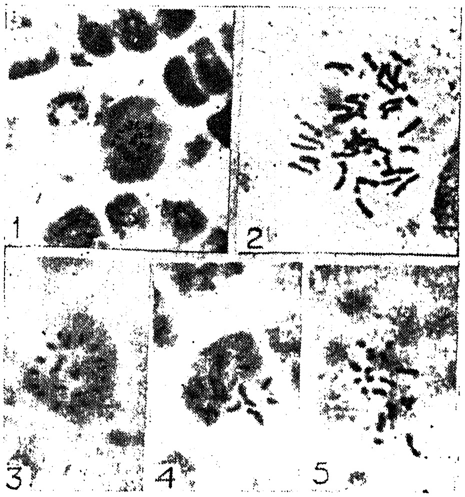
Different species and members of 18 genera were included in the study. Effect of chemical pretreatment, duration of pretreatment temperature as well as nature of fixative and period of fixation on cytological preparation were studied.

For cytological study seeds were raised in earthenware containing sand and soil mixture. When the roots attained 1″ length, 1·5–2 mm apical lengths of roots were taken, washed in water and transferred to pretreatment chemicals of varying concentrations for different durations. Pretreated root tips were fixed in mixture of acetic alcohol (1 : 2) for 1–2 hours. After fixation, they were heated in a mixture of 2% aceto-orcein (N) HCl (9 : 1) solution for 5–6 seconds and kept in the mixture for 6–24 hours. Subsequently, the root tips were squashed in 45% acetic acid sealed and observed. The best schedules for different genera are given in Table I.

It is seen that L-bromonaphthalene proved most suitable for the majority of the species and varieties investigated. The response of chromosomes to chemical pretreatment varied from genus to genus as well as within the species and even within the varieties of the same species.

Pretreatment with a mixture of half saturated solution of aesculine and 0·002 M oxyquinoline (1 : 1) in cold (8°–14° C) proved effective in Hydrocotyloideae and Saniculoideae.

In the genera *Eryngium* and *Centella* however after fixation treatment with saturated solution of pectinase for 15 minutes at 60° C was necessary to get well-defined chromosome morphology.



FIGS. 1-5. Fig. 1. Somatic metaphase with $2n = 22$ chromosomes in *Ammi majus*. Fig. 2. Somatic metaphase with $2n = 38$ chromosomes in *Heracleum wallichii* D.C. Fig. 3. Somatic metaphse with $2n = 24$ chromosomes in *Coriandrum sativum* var. W.B$_4$. Fig. 4. Somatic metaphase with $2n = 22$ chromosomes in *Coriandrum sativum*var. Madras. Fig. 5. Somatic metaphase with $2n = 16$ chromosomes in *Eryngium foetidum*.

Pretreatment with a saturated solution of L-bromonaphthhalene at 8–23° C for 75 minutes to 150 minutes proved satisfactory in members of the subfamily Apioideae.

For leaf tip chromosome preparations, an additional step after pretreatment, *viz.*, keeping in a mixture of Newcomers fluid and Carnoy's fluid (1 : 1) for 2–3 hours was very helpful in clearing the heavy cell content.

For feulgen staining of Umbelliferous materials standardization of time of hydrolysis (with N. HCl at 60° C) was found to be very important.

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* References cited from recent compilation of Federov *et al.* [2].

TABLE I

Optimum pretreatment and fixation schedule of the different genera studied

Name of the genera	Pretreatment			Fixation	
	Pretreating chemicals	Duration of pretreatment	Temperature	Fixative	Period of fixation
Centella L.	Saturated solution of aesculine : oxyquinoline (0·002 M) mixture (1 : 1)	2 hrs.	14° C–16° C	1·2 Acetic alcohol	1 hr. 30 min.
Hydrocotyle L. (2 species)	do.	3–3½ hrs.	do.	do.	do.
Eryngium L.	do.	2 hrs. 15 min.	do.	do.	do.
Sanicula L.	do.	do.	12° C–16° C	do.	do.
Apium L. (7 varieties)	do.	1–2 hrs.	12° C–19° C	do.	do.
Carum L.	Saturated solution of paradichlorobenzene : oxyquinoline (0·002 M) (1 : 1); also saturated L-bromonaphthalene	2 hrs. 15 min. to 2 hrs. 45 min.	14° C–16° C	1·1 Acetic alcohol	do.
Coriandrum L. (20 varieties)	Saturated solution of L-bromonaphthalene	do.	do.	1·2 Acetic alcohol	do.
Heracleum wallichii D.C.	do.	do.	do.	do.	do.
Anthriscus Pers.	do.	do.	do.	do.	do.
Foeniculum L. (5 varieties)	do.	do.	do.	do.	do.
Petroselinum Hoffm.	do.	do	do.	do.	do.
Pastinaca L.	do.	do.	do.	do.	do.
Peucedanum L.	do.	do.	do.	do.	do.
Ferula L.	do.	do.	do.	do.	do.
Daucus L. (8 varieties)	Half saturated, aesculine : oxyquinoline (0·002 M) (1 : 1)	1 hr. and 30 min	8° C–14° C	do.	do.
Ammi L.	do. Also saturated L-bromonaphthalene	1½ hrs. to 2 hrs.	9° C–28° C	do.	do.
Oenanthe L.	do.	2 hrs. and 30 min.	10° C–14° C	do.	do.
Torilis Adans.	do.	2 hrs.	do.	do.	do.

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1. Cauwet, A. M., "Caryosystematique due gene Bupleurum L," In : *The Biology and Chemistry of the Umbelliferae* (ed. Heywood, V. H.), Academic Press, London and New York, 1971, p. 257.
2. Federov, An. A, *Chromosome Numbers of Flowering Plants*, Academy of Sciences, U.S.S.R., 1969 p. 926.
3. Moore, D. M., "Chromosome studies in the Umbelliferae." In Heywood (ed.), *The Biology and Chemistry of the Umbelliferae*, Academic Press, London and New York, 1971, p. 315.

'GLYZAGLABRIN', A NEW ISOFLAVONE FROM *GLYCYRRHIZA GLABRA*

During the course of our investigation on the phenolic components of *G. glabra* roots we earlier reported the unusual occurrence of 2-methylisoflavones[1,2] and liqcoumarin[3]. In the present communication we report the occurrence of a new isoflavone, herein named, glyzaglabrin in *G. glabra* roots.

The extraction and fractionation procedures have been already reported[1]. The ether insoluble fraction was chromatographed over silica gel column. The ethylacetate eluate on preparative T.L.C. purification using benzene gave glyzaglabrin, m.p. 224–5°, gave green colour with alc. $FeCl_3$, the Na-Hg/HCl and gallic acid/H_2SO_4 tests for isoflavones and $-O-CH_2-O-$ groups. λ_{max}^{MeOH} (log ε) 225 (4·10), 295 (3·44), +AlCl_3; 270, 310 nm, + NaOAc; 278, 310 nm. ν_{max}^{KBr} 3400 (OH), 1640 (chelated >C=O), 1550, 1410, 1250, 1240, 1190, 1180, 1070, 1045, 1015, 935 ($-O-CH_2-O-$), *MS*: m/e 298 (M^+, 100%), indicated that compound to have 16 carbon atoms, 297 ($M^+ - 1$, 80%), 286 ($M^+ - CH_2 + 2H$, 50%), 270 ($M^+ - CO$, 20%) 162 (RDA fragment with ring B, 55%), 136 (RDA fragment with ring A, 16·5%), 150 ($162 - CH_2 + 2H$, 25%), 118 ($136 - H_2O$, 52·5%). On acetylation (Ac_2O/C_5H_5N, 24 hours) at room temperature it yielded an acetate, m.p. 218–9°. *P.M.R.* (δ, $CDCl_3$, TMS as internal standard); 2·75 (6 H, s, 2 × $OCOCH_3$), 5·75 (2H, s, 3′, 4′-$O-CH_2-O-$), 6·55 (2H, d, J = 8·5 Hz, $C_{5'}$ – H and $C_{6'}$ – H), 6·90 (1H, d, J = 10 Hz, C_6 — H), 7·10 (1H, s, C_8–H), 7·50 (1H, s, C_2 – H), 8·00 (1H, d, J = 10 Hz, C_5 – H).

The spectral data showed the presence of two hydroxyls and a methylenedioxy group in the isoflavone skeleton. Its U.V. spectrum showed bathochromic shifts with NaOAc and $AlCl_3$[4] characteristic of a free hydroxyl as well as a chelated hydroxyl functions in glyzaglabrin. In addition green ferric reaction indicated he possibility of an hydroxyl being chelated with carbonyl group which could be considered at $C_{2'}$ position. In the P.M.R. the signals at δ 6·90 and 8·00 were ssigned to the ortho-coupled aromatic protons present t C_6 and C_5 positions respectively and two singlets t δ 7·10 and 7·50 were assigned for C_2 and C_8 protons respectively[5]. Thus, one hydroxyl is placed at C_7 posi-on in glyzaglabrin, the remaining hydroxyl and methylenedioxy groups were placed at $C_{2'}$, $C_{3'}$ and $C_{4'}$ posi-on respectively. The doublet at δ 6·55 was assigned for two aromatic protons at $C_{5'}$ and $C_{6'}$. Thus, glyza-abrin was assigned the structure 7,2′-dihydroxy, 3′,4′-methylenedioxyisoflavone. The M.S. also supported this structure as m/e 162 for RDA fragment with ring B and m/e 136 of ring A and the loss of $-CH_2$ group (m/e 150) from the ring B of the RDA cleavage showed that one hydroxyl and a methylenedioxy groups were present at ring B.

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1. Bhardwaj, D. K., Murari, R., Seshadri, T. R. and Radhika Singh, *Phytochem.*, 1976, **15**, 352.
2. —, Seshadri, T. R. and Radhika Singh, *Ibid.*, 1977, **16**, 402.
3. —, Murari, R., Seshadri, T. R. and Radhika Singh, *Ibid.*, 1976, **15**, 1182.
4. Markham, K. R. and Mabry, T. J., *The Flavonoids*. (Harborne J. B., Mabry, T. J. and Mabry, H, eds.), Chapman and Hall, London. 1975, p. 58.
5. — and —, *Ibid.*, p. 68.

INFLUENCE OF NITROGEN NUTRITION AND SHEATH ROT DISEASE ON SUGAR CONTENT OF TWO PADDY VARIETIES

Introduction

In India sheath rot disease of rice caused by *Acrocylindrium oryzae* is assuming serious proportions. Yield losses up to 85% had been recorded by Prabakaran *et al.*[12] due to *A. oryzae* infection in Tamil Nadu. Mohan and Subramanian[8] reported 57% damage due to the disease. It was found that increased nitrogen content led to the susceptibility of the plants (Chien and Chin[3], Mohan and Subramanian[8]). With a view to elicit information on sugar content due to increased N application and its influence on disease reaction in two paddy varieites, the present investigation was carried out.

Materials and Methods

Bhavani, moderately resistant to sheath rot and Kannagi, highly susceptible were raised in the field with three levels of nitrogen, *viz.*, 0, 65 and 100 N kg/ha. The plants were inoculated with the spore suspension (*ca.* 10^7 spores/ml), when they were 80 days old. Suitable controls were also maintained.

Leaf sheath samples were collected for analysis from healthy and inoculated plants, 1, 8 and 15 days after inoculation in the morning. The sheaths were extracted in boiling ethanol (Chandramohan *et al.*[2]).

Reducing sugar and the total sugar of the extract were estimated by Nelson's method[9]. The alcohol-extract was hydrolyzed with 1N H_2SO_4 (Inman[6]) for 30 min. at 49° C and after neutralizing the total soluble sugar content of the sample was obtained

Results

At higher levels of nitrogen, reducing sugars increased in both Kannagi and Bhavani. The quantity of reducing sugar, in general, was slightly higher in Kannagi than in Bhavani. Inoculation caused a slight reduction in reducing sugar level (Table I). The level of non-reducing sugars increased with increased nitrogen application. The quantity of non-reducing sugars was higher in Kannagi than in Bhavani. Inoculation slightly decreased the non-reducing sugar in both the varieties (Table II).

TABLE I

Changes in reducing sugars of rice cultivars due to* Acrocylindrium oryzae *inoculation and nitrogen fertilisation* (mg/g dry weight)

Days after inoculation	Variety	N levels (kg/ha) 0.N H	0.N I	65.N H	65.N I	100.N H	100.N I
1	Kannagi	3·39	3·42 (+0·88)	3·62	3·57 (+1·38)	3·88	4·02 (+3·60)
	Bhavani	3·27	3·27 (0·00)	3·57	3·53 (−1·12)	4·27	4·30 (+0·70)
8	Kannagi	4·46	4·37 (−2·10)	4·74	4·26 (−10·13)	5·06	5·27 (+4·15)
	Bhavani	4·37	4·26 (−2·26)	4·40	3·91 (−11·14)	4·98	4·82 (−3·22)
15	Kannagi	4·81	4·42 (−8·11)	5·13	4·50 (−12·28)	6·46	6·02 (−6·82)
	Bhavani	4·96	4·87 (−1·83)	5·05	4·67 (−7·53)	6·42	5·76 (−10·50)

* Expressed in glucose equivalents.
Figures within the parentheses represent the per cent increase (+) or decrease (−) over healthy.

TABLE II

Changes in non-reducing sugars of rice cultivars due to* Acrocylindrium oryzae *inoculation and nitrogen fertilisation* (mg/g dry weight)

Days after inoculation	Variety	N levels (kg/ha) 0.N H	0.N I	65.N H	65.N I	100.N H	100.N I
1	Kannagi	1·53	1·50 (−1·96)	1·84	1·76 (−4·35)	2·34	2·29 (−2·14)
	Bhavani	1·10	1·01 (−8·19)	1·25	1·36 (+8·80)	2·40	2·41 (+0·42)
8	Kannagi	2·36	2·10 (−11·02)	2·49	2·60 (+4·42)	3·91	3·65 (−6·65)
	Bhavani	2·15	1·80 (−16·28)	1·83	1·76 (−3·82)	2·74	3·00 (+6·00)
15	Kannagi	2·07	2·00 (−3·39)	2·88	2·74 (−4·86)	3·40	3·45 (+1·47)
	Bhavani	1·98	1·50 (−24·24)	2·92	2·42 (−17·13)	3·26	3·01 (−7·61)

* Expressed in glucose equivalents.
Figures within the parentheses represent the per cent increase (+) or decrease (−) over healthy.

Discussion

Generally, the sugar levels in the healthy and inoculated plants are correlated with resistance (Horsfall and Dimond^{5}). In the present study, the levels of both reducing and non-reducing sugars increased in both Kannagi and Bhavani due to increased N application. The quantity of reducing and non-reducing sugars was higher in Kannagi than in Bhavani. High amounts of reducing and non-reducing sugars have been recorded in many plants susceptible to several pathogens (Otani^{11}, Ohata *et al.*^{10}; Jayapal and Mahadevan^{7}). Inoculation with the pathogen showed a reduction in reducing and non-reducing sugar levels in both Kannagi and Bhavani. Ranga Reddy and Sridhar^{13} reported a same trend in rice plants infected with *Xanthomonas oryzae*.

The presence of more sugars in the tissue tends to increase susceptibility to invading pathogens as they serve sources of energy to the pathogen. So *A. oryzae* may be considered to be a "high sugar" disease. In several host parasite interactions, the levels of tissue sugars decrease following infection (Asada^{1}, Dayal and Joshi^{4}). The reduction of sugars in the infected sheaths might be due to the utilization of these compounds by the pathogen and to the decreased synthetic ability of the severely infected leaves (Ranga Reddy and Sridhar^{13}).

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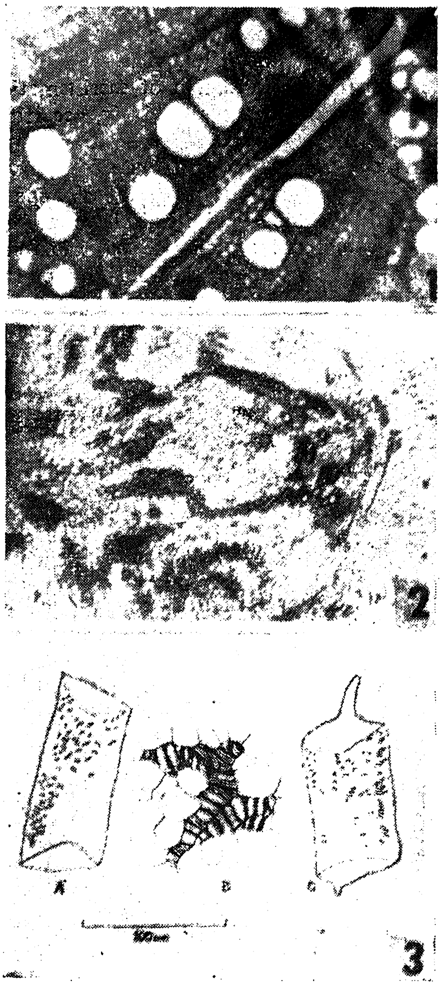
1. Asada, Y., *Ann. Phytopath. Soc., Japan*, 1957, **22**, 103.
2. Chandramohan, D., Mahadevan, A. and Rangaswamy, G., *Indian Phytopath.*, 1967, **20**, 109.
3. Chien, C. C. and Chin, C. L., *J. Taiwan Agric. Res.*, 1970, **19**, 62.
4. Dayal, R., and Joshi, M. M., *Indian Phytopath.*, 1968, **21**, 221.
5. Horsfall, J. G. and Dimond, A. E., *Z. Pflanzenkrankh Pflanzenes Chutz.*, 1957, **64**, 415.
6. Inman, R. E., *Phytopathology*, 1962, **52**, 1207.
7. Jayapal, R. and Mahadevan, A., *Indian Phytopath.*, 1968, **21**, 43.
8. Mohan, R. and Subramanian, C. L., *M.Sc. (Ag.) Thesis*, Tamil Nadu Agric. Univ., Coimbatore, 1976.
9. Nelson, J., *J. Biol. Chem.*, 1944, **15**, 375.
10. Ohata, K., Goto, K. and Kozaka, T., *Bull. Natl. Inst. Agro. Sci., Japan*, 1966, **20**, 1.
11. Otani, Y., *Mem. Fac. Agric. Hokkaido Univ.*, 1953, **1**, 375.
12. Prabakaran, J., Ragunathan, V. and Prasad. N. N. *Annamalai Univ. Agric. Res. Ann.*, 1973, 5, 182.
13. Ranga Reddy, P. and Sridhar, R., *Indian Potash J.*, 1976, **1** (4), 13.

ON THE ATYPICAL TRACHEARY ELEMENTS OF *ACACIA LEUCOPHLOEA* GALLS

THOUGH normality in form and structure is known to be a consequence of regular expression of morphogenetic phenomena controlled by genic or cytoplasmic factors (with an environmental impetus as well) involving a systematic transformation of meristematic cells into specialised cells of the plant body, occurrence of anomalous cell types especially in abnormal growth conditions such as those of galls induced by various organisms^{1} and aseptic culture of tissue^{2} appears to be of interest. Küster^{1} and Mani^{3} have elaborately reviewed the morphology and the possible functions of such anomalous cell types occurring in the instances of galls. Present investigation includes a comparative study of the tracheary elements of normal and galled shoot regions of *Acacia leucophloea* Willd. (Leguminosae).

Temporary preparations of serial transverse sections and macerations (by Jeffrey's method^{4}) of the normal secondary tissues and woody galls of *A. leucophloea* were obtained, and were stained with aqueous toluidine blue. Diffuse porous normal wood of *Acacia* (Fig. 1) exhibited vessel elements of varied dimensions (120–150 × 40–50 μ) with dense alternate pitting, inclined or horizontal end plates (Fig. 3A) and occasionally a tail could be observed (Fig. 3C). The amorphous galls induced by *Haplophragmium ponderosum* Syd., on *A. leucophloea*^{5} in transections revealed radiating, horizontally differentiating vascular system (Fig. 2), composed of shorter and thinner elements (80–90 × 20–30 μ) with annular or reticulate wall thickening (Fig. 3B) with the perforation generally occurring in one or both the terminals along the tangential wall (see also Refs. 6, 7) in addition to various other abnormal cell types. Anomalous tracheary cells have been observed in the early stages of gall development, and they, with the ageing of the gall constitute an anastomosing network. Further, along the peripheral region of the galls, characteristic concentric rings of parenchyma cells were evident, which were gradually transformed into tracheary elements of earlier stated morphology, constituting vascular nests as observed in bacterial galls^{8}.

Distinctness in the morphology of the atypical xylem elements in galls compared with the normal ones of *A. leucophloea* appears significant. Although Wardlaw^{9} attributes the induction of the new tissue to the diffusion of an 'exogenous substance', the reorientation of the morphogenetic process in a parenchyma cell to differentiate into a tracheary element completely differing from the characteristic morphology of the vessel element *of that species* indicates that, possibly the exogenous substance is able to relieve the cells from the correlation-factors of cells. Also, the incidence of isolated tracheary elements in an atmosphere of parenchyma cells recalls the suggestion of Wright[10] and Wareing[11] proposing the possible action of certain cytoplasmic determinants for such differential action.



FIGS. 1–3. Fig. 1. Transverse section of normal wood of *A. leucophloea* (× 200). Fig. 2. Transverse section of the gall of *Acacia* induced by *Haplophragmium* (× 70). Fig. 3. A and C: Vessel elements of normal wood. B: Atypical elements of the gall.

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---

1. Küster, E., *Anatomie der Gallen*, Handb. der Pflanzenanatomie, Ed. K. Linsbauer, 1930.
2. Steward, F. C., Mapes, M. O. and Mears, K., *Amer. J. Bot.*, 1958, **45**, 705.
3. Mani, M. S., *Ecology of Plant Galls*, W. Junk Publishers, The Hague, 1964.
4. Johansen, D. A., *Plant Microtechnique*, McGraw-Hill Book Co., Inc., New York, 1940.
5. Thirumalachar, M. J., *Jour. Ind. Bot. Soc.*, 1941, **20**, 223.
6. Swamy, B. G. L. and Krishnamurthy, K. V., *Phytomorph.*, 1971, **21**, 36.
7. Raman, A. and Devadas, C., *Proc. Indian Acad. Sci.*, 1977 (In press),.
8. Therman, E., *Caryologia*, 1956, **8**, 325.
9. Wardlaw, C. W., *Phylogeny and Morphogenesis*, Macmillan and Co., London, 1952.
10. Wright, S., *Amer. Nat.*, 1945, 79, 289.
11. Wareing, P. F., *Control Mechanisms of Growth and Differentiation*, 1971, **25**, 323.

SURFACE IMPRINTING BY COMMERCIAL PLASTIC

THE use of transparent replicas has long been a convenient method for the study of plant surfaces. Imprints of fossil petrifications have often been prepared with different imprinting materials[2]. The use of such techniques has provided an additional visual aid in epidermal studies too. For the study of epidermal cell development, stomatal behaviour, and the structural details of the cuticle, epidermis, and petrified surfaces different chemicals, mucilage from fruits, latex from the stems, and recently some domestic adhesives have been tried[1,3–8].

The present report describes another simple, cheap and quick method for epidermal imprinting. Photographs are given to elucidate the usefulness and versatility of the method.

Commercial plastic is being used as the imprinting material. The procedure can be described as follows:

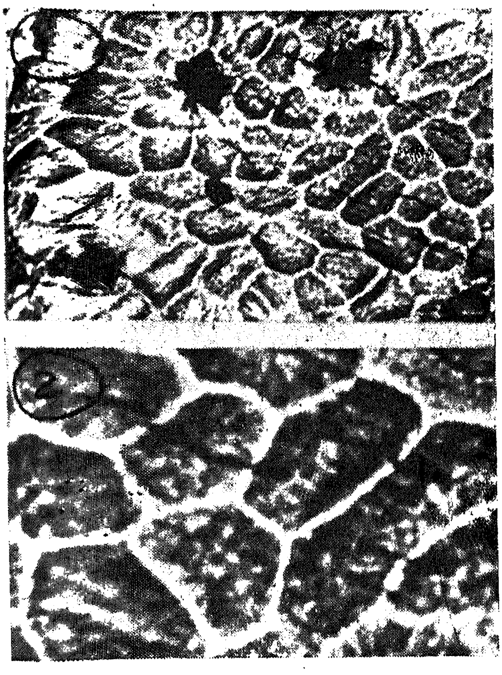
1. Commercial plastic (2%) solution is prepared in toluene at room temperature.

. The surface to be coated is first washed with
water and then with absolute alcohol to elimi-
: the foreign material.

. A drop of the plastic solution is applied on the
surface. The solution spreads by itself and makes
niform coating over the surface. It hardens up
a whitish translucent film when dried.

The film is then peeled off with forceps and
ed on a dry microscope slide with the imprinted
ace upward and covered with a clean cover glass
neans of Quick fix on each corner.

e preparation is now ready for microscopic
ination. The whole procedure is completed
in 5–6 minutes. Results are quite satisfactory
own in Figs. 1 and 2. In addition to the ease
pplication and removal, the plastic has an added
ntage of being non-toxic to the tissues and hence
ted imprints can be obtained from the leaves
u for various physiological studies.



s. 1 2. Fig. 1. Epidermal imprint of the lower
: of *Rosa sinensis* leaf (× 190). Fig. 2. A
n of the same magnified (× 525).

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1. Bhat, R. B., Gangadhara, M., Bhatt, D. C. and Inamdar, J. A., *Curr. Sci.*, 1976, **45**, 344.
2. El-Saadawi, W. E., Badawi, A. A. and El-Awamri. A. A., *Ann. Bot.*, 1976, **40**, 1321.
3. Inamdar, J. A. and Patel, R. C., *J. Micros.*, 1969, **90**, 269.
4. —, — and Bhatt, D. C., *Acta Micros.*, 1970, **70**, 140.
5. Joy, K. W., Willis, A. J. and Lacey, W. S., *Ann. Bot.*, 1956, **20**, 635.
6. Long, F. I. and Clement, F. E., *Am. J. Bot.*, 1934, **21**, 7.
7. Payne, W. W., *Word's Bull.*, 1968, **7**, 6.
8. Shah, G. L. and Gopal, B. V., *Stain Technol.*, 1969, **44**, 123.

COMPARATIVE STUDY OF THE RELATIVE ORDER OF LIGAND FIELD STRENGTH IN COBALTIC COMPLEXES

Temperature independent paramagnetism (χ_p) values of the cobaltic complexes of the type [Coen$_2$ (R) Cl]Cl$_2$ where 'R' is an amine have been reported earlier by the authors[1]. On the basis of Griffith and Orgel's[2] theory, the authors have theoretically calculated the χ_p values and reported[3]. NMR spectra of all these complexes were investigated. Their chemical shift values "σ" are determined[4]. Table I summarises all these values.

Discussion of the Results

One of the conclusions of the Griffith and Orgel's[2] theory is that the χ_p term should decrease as the ligand field strength increases. Therefore assuming χ_p values as a measure of ligand field strength an order of relative ligand field strength for various ligands can be expressed as under:—

1. *Magnetic measurements*

Dicyandiamide > pyridine > ortho-phenetidine > meta-toluidine > meta-chloroaniline > para-anisidine > ortho-chloroaniline > meta-anisidine > meta-phenetidine.

2. *Spectrochemical data*

Dicyandiamide > pyridine > ortho-phenetidine > meta-chloroaniline > para-anisidine > ortho-chloroaniline > meta-anisidine > meta-phenetidine > meta-toluidine.

3. *NMR Data*

Taking chemical shift "σ" values as a direct measure of ligand field strength the order of relative ligand field strength runs as:—

Dicyandiamide > pyridine > meta-toluidine > meta-anisidine > meta-chloroaniline > para-anisidine > ortho-phenetidine > meta-phenetidine > ortho-chloroaniline.

TABLE I

χ_p values and NMR shifts of cobaltic complexes

Sl. No.	Formula of the complex (complexing ligand)	χ_p (Obs.)[1]	χ_p (Calc.)[3]	σ% from NMR data[1]
1.	$[Coen_2(C_2H_2N_4)\,Cl]Cl_2$ (dicyandiamide)	131·73	202·2	−0·713
2.	$[Coen_2(C_6H_5N)\,Cl]Cl_2 \cdot H_2O$ (pyridine)	153·24	208·3	−0·803
3.	$[Coen_2(C_6H_4 \cdot NH_2 \cdot OC_2H_5)Cl]Cl_2$ (ortho-phenetidine)	153·70	208·3	−0·822
4.	$[Coen_2(C_6H_4 \cdot NH_2 \cdot CH_3)Cl]Cl_2 \cdot H_2O$ (meta-toluidine)	166·14	212·4	−0·818
5.	$[Coen_2(C_6H_4 \cdot NH_2 \cdot Cl)\,Cl]Cl_2$ (meta-chloroaniline)	171·12	208·3	−0·821
6.	$[Coen_2(C_6H_4NH_2\text{-}OCH_3)Cl]Cl_2 \cdot H_2O$ (para-anisidine)	186·40	208·3	−0·821
7.	$[Coen_2(C_6H_4 \cdot NH_2 \cdot Cl)Cl]\,Cl_2$ (ortho-chloroaniline)	208·39	210·4	−0·871
8.	$[Coen_2(C_6H_4 \cdot NH_2 \cdot OCH_3)\,Cl]Cl_2$ (meta-anisidine)	208·53	212·4	−0·818
9.	$[Coen_2(C_6H_4 \cdot NH_2\text{–}OC_2H_5)Cl]Cl_2$ (meta-phenetidine)	211·48	212·4	−0·830

It would be interesting to compare the relative order of ligand field strengths obtained by the three methods.

An examination of the results, given above, indicate that there is no complete agreement in the orders of ligand field strengths obtained by the three methods. Among the three methods the NMR is perhaps the most sensitive for establishing the relative order for various ligands since the accuracy of NMR measurements is much greater than the other two methods. For example, the absorption bands for the complexes of pyridine, orthophenetidine and meta-chloroaniline are all at 510 mu; however, the NMR shifts for the corresponding ligands are respectively 0·803, 0·822 and 0·821 for the three compounds. The magnetic method has a distinct disadvantage in the fact that a number of uncertainties are involved in obtaining the computed values by using the diamagnetic susceptibility values of the components in the complex molecule. An exact correlation in the orders of ligand field strengths by the three methods is therefore not possible. For the ligands, dicyandiamide and pyridine the agreement is good, since in every method these two ligands are heading the list. However, for the remaining ligands there is much variation. A somewhat partial agreement is indicated by the following ligands which may be expressed as

Dicyanidiamide > pyridine > meta-chloroaniline > para-anisidine > meta-phenetidine.

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1. Dodwad, S. S. and Datar, M. G., *Curr. Sci.*, 1977, **46**, 7.
2. Griffith, J. S. and Orgel, L. E., *Trans. Farad. Soc.*, 1957, **53**, 601.
3. Dodwad, S. S. and Datar, M. G., *Curr. Sci.* 1977, **46**, 505.
4. — and —, *Proc. Ind. Acad. Sci.* (communicated).

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TWO TERRICOLOUS LICHENS FROM SOUTH WESTERN INDIA

IN the course of our investigations on the lichen flora of Western Ghats, India, we encountered two microlichens growing on calcareous soil in association with *Riccia* sp. at the Purandar Fort and Poona. On microscopic examination, these lichens were identified as *Dermatocarpon hepaticum* and *Endocarpon pusillum*. These two taxa constitute additions to the lichen flora of India and are therefore described and illustrated in this note specially because of their inconspicuous nature and their known distribution in the temperate regions of the northern hemisphere.

1. *Dermatocarpon hepaticum* (Ach.) Th. Fr. in *Nova Acta Reg. Soc. Sci. Upsal*, Ser. 3, **3**: 555, 1861 (Figs. **1**, **2**).

   = *Endocarpon hepaticum* Ach. Kgl. *Vetensk. Akad. Nya Handl.*, 1809, p. 156.

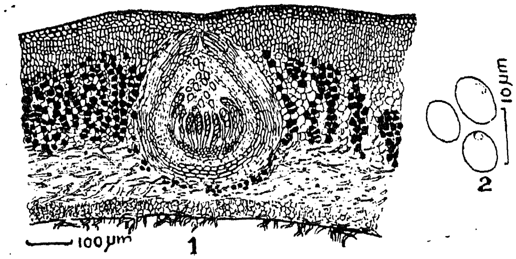
Thallus squamulose, closely adnate; upper surface dark or reddish brown, lower surface pale coloured with a few haptera like rhizoidal hyphae, distinctly corticated on both sides; upper cortex with vertical cell rows, 80–160 μm thick, medulla cellular, hyaline; lower cortex composed of vertical row of cells, thinner than upper cortex; algae mostly in upper cortical layers; perithecia immersed, 300–400 μm in diam., perithecial wall (exciple) thick, mostly colourless but dark brown near the dark ostiole; hymenium I–;

asci cylindrical, unitunicate, shortly pedicellate; ascospores one celled, hyaline, ellipsoidal to subglobose, 4-5 × 8-13 µm in size.

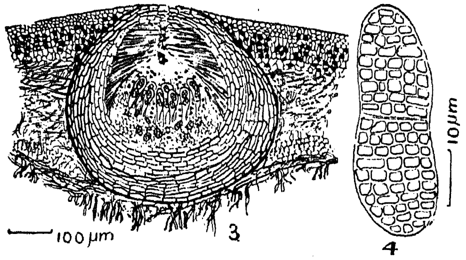
Specimens examined: Poona, *P. G. Patwardhan*—74·314, 315, 316, 317, 318, 319, 320–AMH.

Distribution: Europe, Egypt, Cape of Good Hope, Israel and now India.

2. *Endocarpon pusillum* Hedw. in Descript. et Adumbrat. Muscor. Frondos. 2: 56, 1789 (Figs. 3, 4).



FIGS. 1–2. Fig. 1. *Dermatocarpon hepaticum* (Ach.) Th. Fr. V.S. of thallus through perithecium. Fig. 2. Ascospores.



FIGS. 3–4. Fig. 3. *Endocarpon pusillum* Hedw. V.S. of thallus through perithecium. Fig. 4. Ascospores.

Thallus squamulose, closely adpressed, pale green when fresh, 1·0–2·0 mm broad; squamules 0·4–0·6 mm broad, 400–500 µm thick, corticated on both sides; upper cortex paraplechtenchyamatous, 70–100 µm thick, lower cortex 80–100 µm thick, dark brown to black at lower part; algal layer continuous below upper cortex; perithecia immersed, scattered, 400–500 µm in diam., hymenium hyaline I–, containing algae; perithecial wall thick, hyaline; asci cylindrical to clavate, unitunicate, bispored; ascospores oblong to ellipsoid, slightly constricted at the centre, muriform, hyaline, ends obtuse, 12–26 × 35–60 µm in size.

Specimens examined: Purandar Fort, *M. B. Nagarkar*—77·802, 813—AMH.

Distribution: North America, Europe, Pakistan and now India.

Remarks: This genus is characterised by (muriform ascospores and presence of algae in the hymenium. It was previously reported from Indian subcontinent—from Gujranwala, Punjab, Pakistan—by Smith[2] and Chopra[1].

We are thankful to Prof. M. N. Kamat, M.A.C.S. Research Institute, Pune 4 and Dr. Mason E. Hale, Smithsonian Institution, Washington, D.C., U.S.A., for their keen interest in this work. The work was supported by a National Science Foundation, Washington, D.C., U.S.A. grant (GF–36745) to the M.A.C.S. Research Institute, Pune 4.

Department of Mycology and Plant Pathology,
Maharashtra Association for the Cultivation of Science,
Research Institute, Pune 411 004,
India, *September* 10, 1977.

P. G. PATWARDHAN.
A. V. PRABHU.
M. B. NAGARKAR.
URMILA MAKHIJA.

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1. Chopra, G. L., *Lichens of Himalayas*, Punjab University, Lahore, 1934, p. 14.
2. Smith, A. L., *Trans. Brit. Mycol., Soc.*, 1931, **11**, 128.

A NEW REPORT ON THE COPROPHILOUS ASCOMYCETES FROM INDIA—I

Mycoarachis inversa

DURING the investigation of the Coprophilous Ascomycetes from India, the author collected *Mycoarachis inversa* Malloch and Cain, on the dung of buffalo. This genus was erected by Malloch and Cain[1] to accommodate a cleistothecial fungus in the family Pseuderociaceae. The characteristic features of the fungus as reported by Malloch and Cain[1] are: Non-stromatic, non-ostiolate, sub-globose to globose ascocarps; colour of the ascocarp varies from olive green to black; asci 8-spored, irregularly disposed, subglobose to globose; ascospores two-celled, hyaline, smooth, without germ pore or slit.

HABITAT: On buffalo dung, Delhi, April 8, 1976, Meera Sharma (RUBL No. 2565).

The author is grateful to Prof. B. Tiagi and Dr. B. L. Jain for encouragement and suggestions, University of Rajasthan for awarding scholarhip.

Department of Botany,
University of Rajasthan,
Jaipur 302 004, India, *June* 30, 1977.

MEERA SHARMA.

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1. Malloch, D. and Cain, R. F., *Canad. Jour. Bot.*, 1970. p, 48.

SHORT SCIENTIFIC NOTES

Leptosphaeria herpotrichiodes De Not., A New Record From India

Leptosphaeria herpotrichiodes de Not. was obtained from dead twigs of *Saccharum munja* Roxb., floating in the river Ganges at Sahibganj, Bihar and Farakka, West Bengal. Earlier this fungus has been reported from Italy[2], Rome[3], U.S.A.[1], Bulgaria[4] and Switzerland[5], where it is known to cause foot rot disease of wheat and other cereals. The morphological details of the fungus are as follows:

Perithecia superficial, black, globose, ostiolate, 150–300 μ in diameter; asci subclavate to cylindric, bitunicate, octosporous, 77·7–87·0 × 14·8–16·7 μ; ascospores brown, biseriate, fusoid, 6–8 septate, 25·9–33·3 × 7·4–9·3 μ; paraphyses absent.

The specimen is deposited at CMI, Kew, England, under accession number IMI 213071.

The authors are grateful to Drs. Anthony Johnston and A. Sivanesan of CMI, Kew, England, for their help in identification of the fungus.

P.G. Botany,
Bhagalpur University,
Bhagalpur 812 007,
September 23, 1977.

K. S. BILGRAMI.
M. A. RIZWI.

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1. Shoemaker, R. A. and Tyler, L. J., *Abs. in Canad. Phytopath. Soc.*, 1954, **22**, 17.
2. Silbilia, C., *Bull. Staz. Pat. Veg. Roma*, 1957, Ser. 3 (15), 335.
3. —, *Ibid.*, 1960, Ser. 3 (18), 191.
4. Todorova Mme, V., *Bull. Plant. Prot. Sofia*, 1957, **6**, **4** (12), 15.
5. Zogg, H., *Schweiz. Landw. Mh.*, 1950, **28** (7), 250.

On a New Record of Black Ruby, *Puntius nigrofasciatus* (Günther) from India (Cypriniformes: Cyprinidae)

The Black Ruby, *Puntius nigrofasciatus*, is a small beautifully coloured freshwater fish which is hitherto known only from Southern Ceylon (Sri Lanka)[1–3]. It can be easily distinguished from other species of *Puntius* by three vertical black bands on the body, serration on the last undivided dorsal ray, absence of barbels and a complete lateral line with 20–22 scales[1,3].

Recently, while working out a collection of riverine fishes of Western Ghats, I came across six examples of *Puntius nigrofasciatus*, measuring 38·0 to 45·0 mm in total length, collected from a rivulet in the forest area of Ponda in Goa. The present find, therefore, extends the distributional range of this species considerably northwards in the Western Ghats of India. Besides this species, eight other Indian species of *Puntius* are also known to occur in Sri Lanka[4].

Since the genus *Puntius* contains members of only primary freshwater fishes[5], the occurrence of common species between India and Sri Lanka supports the view that a land connection must have existed between India and Sri Lanka in the geological past. *P. nigrofasciatus* resembles *Puntius ticto* (Ham.) which is widely distributed all over India and adjacent countries[1,4]. The fact that *P. nigrofasciatus* is absent in the Himalayan drainages[6], it appears probable that it evolved in the Western Ghats from a stock of *Puntius* sp. resembling *P. ticto* and subsequently migrated to Sri Lanka through the land connection which presumably existed then between India and Sri Lanka. This view is also supported by the fact that freshwater fish fauna of India is believed to have originated in South-Chinese region and subsequently migrated to the Himalayas and to peninsular India, etc[5].

The author is thankful to Dr. B. K. Tikader, Deputy Director of this station, for the facilities provided.

Zoological Survey of India,
Western Regional Station,
Poona 5, *July* 23, 1977.

G. M. YAZDANI.

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1. Day, F., *Faun. Brit. Ind. Fish.*, 1889, **1**, 328.
2. Mendis, A. S., *Fishes of Ceylon*, Fisheries Research Station, Department of Fisheries, Ceylon, 1954. Bulltein No. 2, 93.
3. Sterba, G., *Freshwater Fishes of the World* (Translated and Revised by Dennys Tucker), Vista Books, London, 1962, pp. 288, 289.
4. Yazdani, G. M. and Rao, M. B., *J. Bombay Nat. Hist. Soc.*, 1976, **73**, 171.
5. Menon, A. G. K., *Curr. Sci.*, 1973, **42**, 553.
6. —, *J. Zool. Soc. India*, 1963, **14**, 23.

REVIEWS

Supplement to Duthie's Flora of the Upper Gangetic Plain and of the Adjacent Siwalke and Sub-Himalayan Tracts. By M. B. Raizada. (Bishen Singh, Mahendra Pal Singh, Dehra Dun), 1976. Pp. vii + 355. Price not given.

This book is an outcome of the author's realisation of the imperative need for bringing Duthie's Flora up-to-date. The author has maintained as far as possible Duthie's work in its original form and has taken pains to present the whole literature on the new species of the upper Gangetic plain in detail. He has rightly stated that "the supplement does not claim to be perfect nor complete; but every effort has been made to present as completely and reliable a list of plants of the upper Gangetic plain as was possible with the means available". A book of this type is always most welcome, because Flora's are referred to constantly by the teachers, students and research workers both in the class room and in the field. In a developing discipline where the concepts and nomenclatures appear to change often, supplements and new editions which include newer plant species and the latest nomenclature of the old species will be highly beneficial for all the workers in the field.

The book primarily deals with the enumeration of the plant species available in the upper Gangetic plain. It would have been much more useful and helpful had there been a taxonomic key of the genera and the species particularly for those which have been described anew. Also illustrations of some of the important species could have been included.

The book deserves to be patronised by the systematic botanists as it will be very useful at both the Graduate and Post-Graduate level of teaching and research.

R. A. Srimathi.

The Mathematical Theory of Electricity and Magnetism. By D. K. Sinha and R. Bandyopadhyaya. (Published by Macmillan Company of India), Pp. 525. Price Rs. 45·50.

This book is supposed to be dealing with the mathematics of the theory of electricity and magnetism. However a subject like this cannot avoid physics altogether. Wherever the authors try to talk about the physical aspects of the various problems, they have given wrong statements and interpretation with a few exceptions. Even the mathematical aspect of the book is poorly done. The end product is neither good mathematics nor good physics. The students would be wasting their money in purchasing this book as many better texts are available in the market.

Most chapters start with a historical background of the subject. As a consequence the reader gets a feeling that the authors are going to give a very serious minded treatment, but soon after that one begins to get disappointed. If the teachers and students do not observe caution they are going to end up with learning wrong concepts. In the early part of the book mention is made of MKS units before the introduction of natural units. The authors should not have talked about MKS units, (because in its absence it would have simply implied the use of natural units only) as no chapter on the units appears in the book. There is a big chapter covering about 46 pages on special functions. But a very negligible part of it has really been applied in solving problems. Where is the need for such a chapter particularly when the material given therein can be found in many other books?

Even simple definitions of pulse, and standing waves have been given incorrectly or without any reference to the foregoing. I could go on and list number of other basic errors but that is not my job to correct a book written by others. The book has no value for the mathematics students as it contains no unsolved problems on the subject.

Som Krishan.

Soil Science, Chemical Properties No. 5 in Text Book Series in Agricultural Chemistry. By. Dr. A. Sankaram. (Published by The Bangalore Printing and Publishing Company Limited, Mysore Road, Bangalore 560018), 1977. Pp. 184. Price: Rs. 16/-.

Among the environmental components of greatest significance in agricultural production, soil and water play a major role. A myriad of chemical reactions occur in soil under the influence of agricultural inputs and management practices. Fertilizer, pesticide and microbial inoculation technologies are constantly advancing and these are applied to soil for enhanced crop production. In order to assess the contribution from each of these factors, a thorough understanding of the chemical transformations in soil is essential for students of Agricultural Sciences.

In the book, aspects of chemical composition of soil, structure and properties of inorganic and organic constituents of soil have been dealt with in an elaborate manner laying emphasis on the methods of analysis and the concepts involved. For a proper understanding of soil chemistry, the most reactive substances, *viz.*, clay and soil organic matter deserve to be discussed from all aspects and this has been done giving sufficient tabulated material and structural diagrams. Illustrations of the type of spherical bodies with cut faces making three-dimensional coordination to convey the idea of clay mineral structure are widely used in literature and such types of figures are not given in the book.

Haworth and Flaig have elucidated the structure and composition of humic substances and reference to their work is lacking in the book. Although reference to recent literature on soil acidity and liming is made, findings reported under International Soil Testing Programme issued by North Carolina Agricultural Experiment Station do not appear to have figured in the book. There are a few more errors apart from those listed in the errata. The indexing of important topics covered in the text should have been a little more imaginative and elaborate.

In spite of the few shortcomings, useful data from Indian Soils is collected and presented. Quantitative data presented in the form of equations, tables and curves are quite handy for interpretations and end-use application for interested students of soil science.

P. B. DESHPANDE.

Application of Vegetation Science to Grassland Husbandry (*Handbook of Vegetation Science*, Vol. 13). Ed. W. Krause. (Dr. W. Junk, b.v. Publishers, the Hague, Netherlands), 1977. Pp. xx + 550, 60 figs., 10 photographs and 10 tables. Price : Dutch Guilders 150.00.

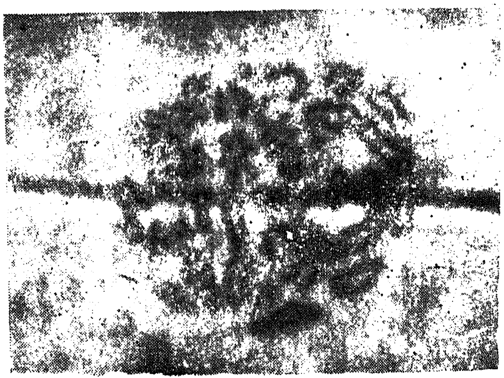
This is the fourth volume published so far in the *Handbook of Vegetation Science* series, and like the other volumes constitutes a useful addition to the scientific literature. However, as with all such edited volumes, the subject-matter receives a rather uneven coverage, with wide variation in the depth and thoroughness with which a given topic is revieved. The primary emphasis is on phytosociology, and there are papers on phytosociology of the grasslands of Canada, of Greenlands, of Mediterranean basin, of Alpine regions and the African range lands. There is a contribution to plant productivity and leaf area of grasslands, and reviews of game and domestic animals on African grasslands and reindeer pastures.

The articles particularly likely to interest the Indian readers are a review of the analysis and management of tropical grazing lands by R. O. Whyte and a review of farming regions in the tropics by B. Andreae. Whyte, in an authoritative review of over a hundred pages, traces the history of tropical grasslands. Many of them, including all the grasslands on the Indian sub-continent, are secondary communities created by human pressures. These grasslands, created in the first place through overexploitation of the climax vegetation, are being further degraded all over India through continued overexploitation. At the present, we seem entirely helpless to deal with the situation except in small pockets where commercial fodder cultivation can be taken up. Andreae's review of tropical farming systems is a brilliant exposition of the ecological basis of tropical agriculture. He demonstrates that the optimum farming systems tend to be of the same physiognomy as the climax vegetation. Thus plantations are the optimal farming systems in heavy rainfall regions, particularly on the slopes ; grazing lands managed at moderate herbivore densities, the optimum farming systems in semi-arid regions and so on. Andreae's arguments deserve careful consideration in the process of land use planning, and so do Whyte's. The book therefore contains significant material not only for professional ecologists, but also for those concerned with larger issues of natural resource management.

MADHAV GADGIL.

ERRATA

With reference to the note "An Improved and Simple Leucocyte Culture Technique for Chromosomal Preparations from Albino Rat" by Usman M. Adhami, S. Tariq M. Haqqi (*Curr. Sci.*, 1977, Vol. 46, p. 502) : In place of Fig. 1, published, which is incorrect, the following figure has to be substituted. The authors regret for the error.



EDITOR : Gross negligence of this type by the authors is strongly resented.

6727/77. Published by Prof. M. R. A. Rao, Current Science Association, Bangalore-560 006
and Printed at The Bangalore Press, Bangalore-560 018.

AN ALTERNATIVE STRUCTURE FOR DNA AND ITS RELEVANCE TO DNA SUPERCOILING

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ABSTRACT

A space-filling (CPK) model of an alternative structure for DNA is given. The structure consists of two strands of opposite polarity each having alternating right and left-handed helical segments. Each segment is approximately five base-pairs long. The strands are held together by Watson-Crick base pairing. Such a structure avoids intertwining and thus offers a natural solution to the problem of a strand separation during replication. In addition, the axes of consecutive helical segments could trace a curve, facilitating supercoiling. The overall energetics of this structure favour it over existing models.

TWO possible alternatives (Type I and Type II) to the classical structure of DNA (B form) were recently proposed by us[1]. These structures maintain Watson–Crick base pairing[2] but avoid intertwining of the two anti-parallel strands. Investigators from New Zealand[3] simultaneously proposed a possible structure for DNA which is very similar to our Type I structure. Both the Type I and Type II structures contain alternating right- and left-handed helical segments, each approximately five base pairs long. In the Type I structure all the sugars in a strand point in the same direction whereas in Type II sugars in alternate segments point in opposite directions. One important property of both these types of structures is that there is no intertwining of the two strands as in the double helix.

We have now made further investigations on structural features of both the Type I and Type II models. On attempting to build space-filling (CPK) models of these two structures, we found that this could be done only with Type II and not with Type I. A CPK model of the type II structure is shown in Fig. 1. A more detailed analysis of both the Type I and Type II conformations has shown that the Type II structure is energetically more favourable. In our opinion these results rule out the possibility of our Type I structure and as well as that proposed by Rodley *et al.*[3]. We discuss here some properties and implications of the Type II structure.

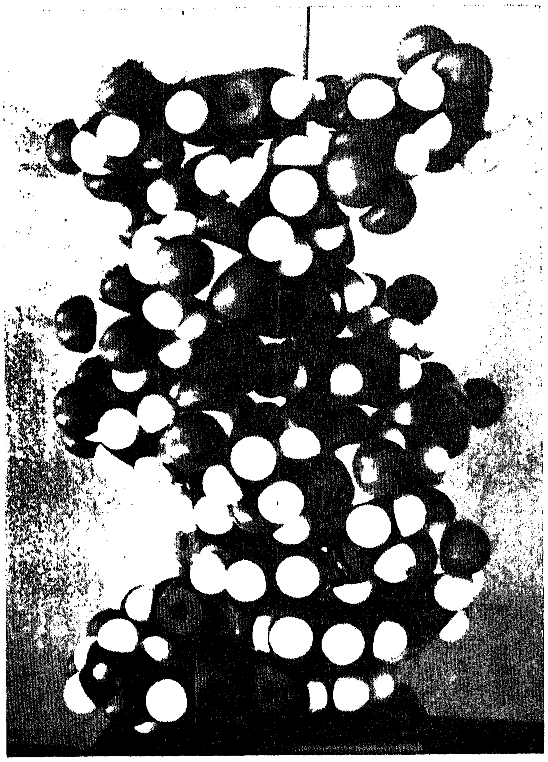

FIG. 1. A space-filling (CPK) model of the Type II structure.

As mentioned earlier, in the Type II structure, sugar residues in alternating segments along a strand point in opposite directions, each of the two strands having alternating right- and left-handed helical segments approximately five base pairs in length. As a conseqence, each strand has bends or folds at places where helical segments of opposite sense are linked. These bends impart a significant amount of flexibility to the polynucleotide, because one has three degrees of freedom to define the relative orientations of two successive segments in terms of their axes. The three degrees of freedom as shown in Fig. 2 are : (*i*) a finite twist, θ, in the coiling of successive helical segments, but maintaining the same helical axis. (*ii*) a lateral displacement, d, of the axes of two consecutive segments, and (*iii*) a relative tilt, α, of the axes of successive segments. We have found that θ for 10 base pairs is given by $0 < \theta \leqslant 30°$ to $40°$. Such a twist will result in a major coiling of the duplex with a minimum number of 90 to 120 base pairs in a repeat of the major coil. The lateral shift, d, always maintains the vertical separation of base pairs at 3·4 Å. The relative tilt, c, allows the base pair separation at the bend to vary from 3·4 to 4·0 Å.

We have made use of one or all of the above three degrees of freedom to generate supercoiled structures of DNA without breaking any hydrogen-bonded base pair in this process. The resulting duplex requires no additional backbone strain or unfavourable base stacking. Thus, by keeping the axes of the segments parallel (*i.e.*, $\alpha = 0$) but displaced by distance d (< 1 Å) between alternate segments and with a finite twist θ ($< 30°$), one can generate supercoiled and looped DNA duplexes of SV40 DNA, with dimensions similar to those observed for SV40 DNA[4]. In other words, supercoiling of DNA can occur in this model without an increase in the free energy of the structure.

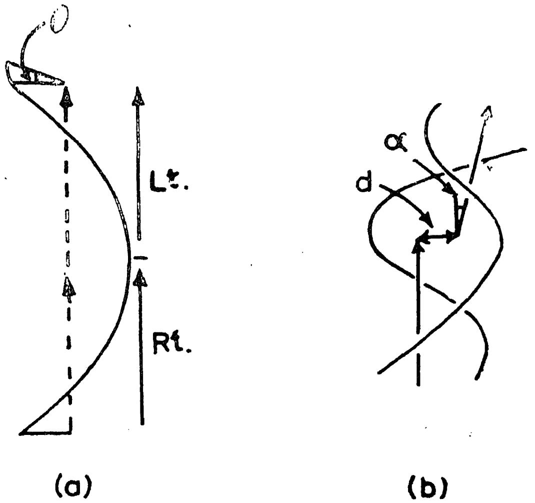

FIG. 2. The three degrees of freedom to define the relative orientation of two consecutive segments of opposite sense. θ, the angle of twist for 10 base pairs about a common helix axis; d, lateral displacement of the axis; α, angle of tilt between axes of two successive segments (*see* text for details).

Let us consider whether this flexibility of the Type II structure could provide a possible solution to the compression of DNA within chromatin[5]. It has been known that the classical double helix is unable to withstand such compression without introducing abrupt discontinuities in the helix[6]. On the other hand, our Type II structure permits the construction of a model of chromatin in which a DNA duplex of diameter 20 Å is wrapped around a core of radius 30 Å, with a pitch of 100 Å for 100 base pairs (*see* Fig. 3). This does not demand any destabilization due to destacking, breaking of hydrogen bonds or steric strain. The octamer[7] histone core of radius 30 Å can readily be accommodated in the inner core of such a supercoil. It, therefore, appears that the inherent flexibility of the Type II structure could result in the initiation of supercoiled structures of DNA following energetically allowed folds. The details will be published elsewhere.

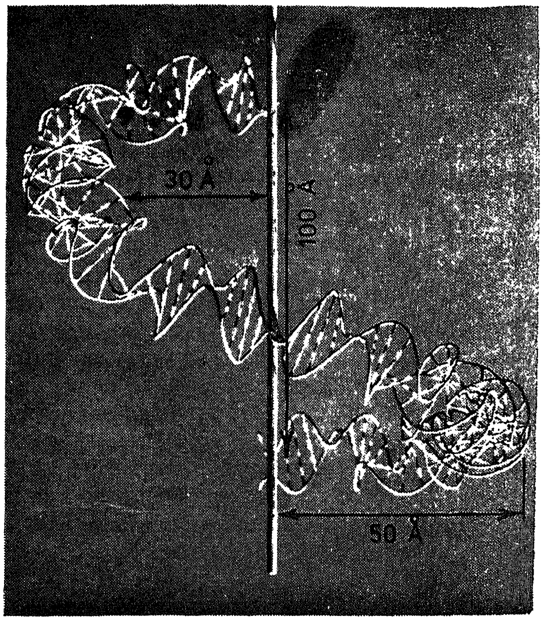

FIG. 3. A wire model of supercoiling of DNA in chromatin. A duplex of DNA of diameter 20 Å is bent to give a radius of curvature of 30–50 Å to wrap around a nucleosome core of diameter 60 Å. Figure shows one turn of the left-handed supercoil with a pitch of 100 Å for 100 base pairs. Neither unfavourable base stacking nor breakage of hydrogen bonds is involved is building the supercoil.

ACKNOWLEDGEMENTS

We thank Prof. H. Sharat Chandra and Dr. V. Nanjundiah for discussions. Financial support from Department of Science and Technology, Government of India, is acknowledged. N. P. thanks the University Grants Commission for a fellowship.

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1. Sasisekharan, V. and Pattabiraman, N., *Curr. Sci.*, 1976, 45, 779.
2. Watson, J. D. and Crick, J. H. C., *Nature*, 1953, 171, 737.
3. Rodley, G. A., Scobie, R. S., Bates, R. H. T. and Lewitt, R. M., *Proc. Natl. Acad. Sci.*, USA, 1976, 73, 2959.
4. Watson, J. D., In : *Molecular Biology of the Gene*, 2nd Edition, (W. A. Benjamin, Inc.. Menlo Park, California, USA), 1970, p. 601.
5. Baldwin, J. P., Boseley, P. G., Bradbury, M. and Ibel, K., *Nature*, 1975, 253, 245.
6. Crick, F. H. C. and Klug, A., *Ibid.*, 1975, 255, 530.
7. Weintraub, H., Worcei, A. and Alberts, B., *Cell*, 1976, 9, 409.

ON THE OCCURRENCE OF PRIESKAITE FROM THE IRON STONE FORMATION, BABABUDAN HILLS

R. V. RAGHAVENDRA, G. V. ANANTHA IYER AND T. R. NARAYANAN KUTTY

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ABSTRACT

A new variety of amphibole asbestos, prieskaite, has been observed in the iron stone formation of Doopadagiri area, Bababudan hills. The chemical composition corresponds to $(Ca_{1\cdot85}\ Na_{0\cdot08})\ (Mg_{0\cdot87}\ Fe^{2+}_{2\cdot55}\ Fe^{3+}_{0\cdot46}\ Al_{0\cdot37})\ SiO_{8\cdot1}\ (OH)_{2\cdot4}$. Prieskaite is formed by the intermixing of basic rocks with the ferruginous quartzites and subsequent metamorphic reactions.

TWO varieties of amphibole asbestos, crocidolite and amosite have been reported from the banded iron ore formations of Bababudans, chiefly confined to Attigundi and Doopadagiri area[1]. Bands of prieskaite have been noticed by the present authors in place of amosite asbestos on the escarpment as well as the dip slopes near the Doopadagiri coffee estate, North Bababudans. The significance of this occurrence, and the mineral chemistry are reported in this communication.

Prieskaite, first discovered by Cilliers[2] in Koega, mine, South Africa, is ferroactinolite with high Fe/(Fe+Mg) ratio. Only a few such actinolite asbestos occurrences are known in the world. The cross fiber bands of prieskaite in iron stones of Doopadagiri vary in width from 0·2 to 0·5 cm (Fig. 1), set at close intervals. The asbestos is greenish white and has low to medium tensile strength. It is often interspersed in bands of quartz, with comb structure. In the banded iron ore formations, are clays, mostly ferronontronite (Table I) derived from weathering of preexisting basaltic rock. Prieskaite asbestos is restricted to the bands spatially associated with basic rocks (metabasalts).

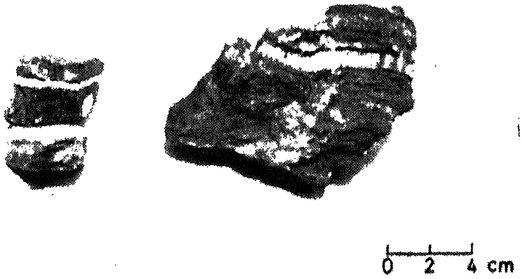

FIG. 1. Cross-fibers of prieskaite in ferruginous quartzite.

TABLE I

Chemical composition of minerals

	1	2	3	4
SiO_2	54·62	52·16	50·72	49·24
TiO_2	0·10	0·27	0·18	0·07
Al_2O_3	2·11	1·82	5·86	5·69
Fe_2O_3	4·12	3·91	0·43	29·78
FeO	20·46	21·38	13·28	0·12
MnO	0·16	0·20	0·09	0·03
MgO	3·86	5·92	15·91	0·64
CaO	11·58	10·99	10·21	2·18
Na_2O	0·26	0·20	0·60	0·14
K_2O	0·10	..	0·16	0·06
H_2O^+	2·42	2·61	2·19	7·69
H_2O^-	0·12	0·18	0·44	13·21
Total	99·91	99·64	100·07	99·85

Number of ions calculated on the basis of 24 (O), 20 (O) atoms and 4 (OH)

	1	2	3	4
Si	8·126	7·917	7·313	7·012
Al^4	..	0·084	0·687	0·988
Al^6	0·369	0·241	0·308	0·132
Ti	0·012	0·038	0·025	0·003
Fe^{3+}	0·461	0·446	0·047	3·291
Fe^{2+}	2·546	2·714	1·692	0·010
Mg	0·866	1·339	3·420	0·210
Mn	0·020	0·026	0·011	0·015
Ca	1·849	1·790	1·580	0·812
Na	0·075	0·059	0·168	0·010
K	0·019	..	0·029	0·007
(OH)	2·404	2·645	2·107	4·064

(1) Prieskaite; (2) Prieskaite; (3) Actinolitic hornblende; (4) Ferronontronite.

MINERAL CHEMISTRY

The chemical formula of the prieskaite reported from Doop dagi i is $(Ca_{1\cdot85}Na_{0\cdot08}\ Fe^{2+}_{2\cdot55}\ Mg_{0\cdot}\ Fe^{3+}_{0\cdot47}\ Al_{0\cdot37})Si_{8\cdot}O_{22}(OH)_{2\cdot4}$, (Table II) indicating that it belongs to tremolite-actinolite series. In contrast, calcic amphiboles from the associated basic rocks have compositions intermediate between actinolite and hornblende with $Al^{4} = 0\cdot684$ and total alkali $= 0\cdot197$ atoms/formula. The 100 Fe/(Fe + Mg) ratio is much lower (around 33) than that of the prieskaite (71–78) which is suggestive of the chemistry of the host rocks.

TABLE II

Chemical composition of whole rocks

	1	2	3
SiO_2	32·12	53·01	43·16
TiO_2	0·21	0·18	0·23
Al_2O_3	0·76	10·79	8·91
Fe_2O_3	53·17	6·42	21·68
FeO	11·03	15·12	16·82
MnO	0·18	0·21	0·28
MgO	1·31	3·45	3·89
CaO	..	7·21	3·74
Na_2O	0·34	0·82	0·71
K_2O	0·11	0·27	0·18
H_2O^+	..	1·81	0·96
H_2O^-	0·10	0·28	0·12
Total	99·33	99·57	100·68

1. Banded ferruginous quartzite.
2. Basic rock.
3. Prieskaite bearing rock.

It is difficult to obtain the exact composition of the banded iron stones because of the varying width of the component bands. In general, they have higher oxides of iron (Fe_2O_3 + FeO = 40–60% by wt.) and SiO_2 (25–35% by wt.). When the bands contain magnesioriebeckite (bababudanite) and aegirine[3,4], alkali (3–6% by wt.) and magnesia (2–5% by wt.) contents are higher. The composition of the basic rock varies with its association. When plotted in MgO-total Fe-alkali diagram, the rock composition has affinity towards tholeiitic basalt with higher CaO, Al_2O_3, MgO and alkali. Composition of the host rock of prieskaite asbestos does not correspond to any of the known mafic rocks. It has lower CaO, Al_2O_3, MgO and alkali and very high iron. It is evident that prieskaite is a product derived by the contamination of the basic rock with iron stones.

The IR spectrum of prieskaite is given along with nonfibrous actinolite in Fig. 2. X-ray powder patterns were taken. The unit cell parameters obtained, $a = 9\cdot87$ Å, $b = 18\cdot13$ Å, $c = 5\cdot29$ Å and $\beta = 104°\ 59'$, tally with the reported values for actinolites[5].

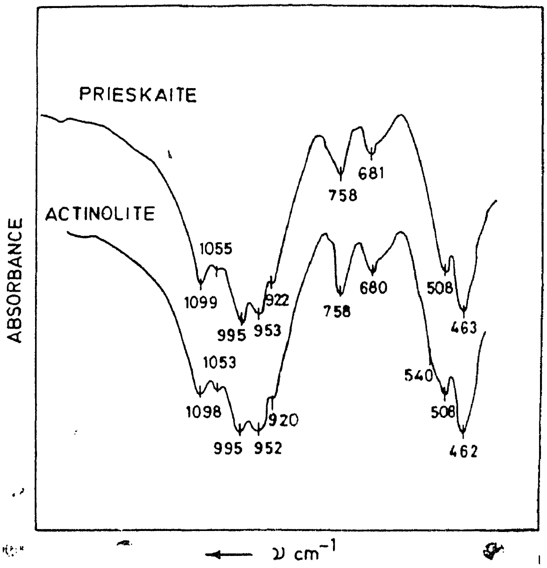

FIG. 2. IR spectra of prieskaite and actinolite.

DISCUSSION

Controversial views are expressed on the mode of formation of iron stones of Karnataka craton; one view is that they are metamorphic products of the basic lava flows[6]. It has also been postulated that ferromagnesium minerals are of igneous parentage which by subsequent weathering and alteration gave rise to iron oxides and silica. A number of investigators[7,8] hold the alternative view that iron formations are of sedimentary origin, the constituents being derived from the land masses by the process of weathering, transportation and deposition in a basin. The basic flows and schists must have constituted the floor of the basin and subsequent diastrophism converted the iron formation into the crescent shaped ranges. However, the origin of magnetite and iron rich silicates in these banded iron ore formations is controversial. The variation in Na_2O, CaO contents of different horizons and formation of the three different amphibole asbestos are difficult to explain. There are a number of alternative explanations put forward for the source of Na_2O and origin of sodic amphiboles and amosite[9-11].

It is evident from the association that the prieskaite is formed in horizons where the basic rocks come in contact with ferruginous quartzites. Necessary calcium for its formation is derived from the basaltic rock. Prieskaite can be regarded as the product of metamorphic reaction such as

$$\begin{array}{l} \mathrm{Ca\,(Mg,Fe)\,Si_2O_6 + 2Fe_3O_4 + 6SiO_2 + H_2O} \\ \quad \mathrm{cpx} \qquad\quad + \;\; \mathrm{mt} \;\; + \;\; \mathrm{qz} \;\; + \mathrm{Water} \\ = \mathrm{(CaFeMg)_7\,Si_8O_{22}\,(OH)_2}, \text{ Prieskaite.} \end{array}$$

This reaction suggests that clinopyroxene (cpx) of original basic rock reacted with the constituents of iron stones under appropriate fluid pressures resulting in the formation of prieskaite. The iron-rich phase is considered as Fe_3O_4; however the equation may be rewritten in terms of Fe_2O_3 as well, assuming appropriate oxygen fugacity. This reaction can take place under P–T conditions of green schist facies.

The development of asbestiform morphology may be attributed to the role of shearing stress, directive pressures or to minor chemical impurities. The formation of cross fibers of prieskaite may as well be related to the control exerted by the parallel surfaces in individual seams during crystallization[12].

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1. Radhakrishna, B. P. and Venkoba Rao, N., *Geol. Soc. Ind. Bull.*, 1967, 4, 99.
2. Cilliers, J. J., *D.Sc. Thesis*, Pretoria, 1961 (unpublished).
3. Raghavendra, R. V., *Ph.D. Thesis*, Indian Institute of Science, Bangalore, 1976 (unpublished).
4. Narayanan Kutty, T. R., Anantha Iyer, G. V. and Ramakrishnan, M., *Curr. Sci.*, 1974, 43, 1.
5. Lyon, R. J. P., *NASA TN-1871*, 1963, p. 87.
6. Smeeth, W. F., *Mysore Geol. Dept. Rec.*, 1908, 9, 85.
7. Pichamuthu, C. S., *J. Geol. Soc. Ind.*, 1974, 15, 1.
8. Sampath Iyengar, P., *Mysore Geol. Dept. Rec.*, 1908, 9, 59.
9. Cilliers, J. J. and LeRand Genis, J. H., *Geology of Some ore Deposits of Southern Africa*, Ed. Houghton, S. H., Geol. Soc., South Africa, 1964.
10. Pichamuthu, C. S., *Proc. Ind. Acad. Sci.*, 1950, 31, 191.
11. Hall, A. L., *Mem. Geol. Surv.*, South Africa, 1930, p. 12.
12. DuToit, A. L., *Trans. Geol. Soc.*, South Africa, 1945, 48, 16.

SOME REPRODUCTIVE ANOMALIES IN THE INDIAN RUFUS HORSE SHOE BAT, *RHINOLOPHUS ROUXI* (TEMMINCK)

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ABSTRACT

The female genitalia of the bat, Rhinolophus rouxi, exhibits pronounced physiological asymmetry, the right ovary normally releasing the ovum during each cycle and the right uterine cornu carrying the pregnancy. Among 285 pregnant females examined for the present study two presented unique features. One female had monozygotic twin embryos at the unilaminar blastocyst stage of development in the right uterine cornu. In another specimen both ovaries had a corpus luteum each, and there was an embryo in each uterine cornu, but the embryo in the right cornu was more advanced in development than the one in the left cornu. It is suggested that the retarded growth of the embryo in the left cornu is due to the development of this embryo in an abnormal environment with deficient hormonal and nutritional levels.

INTRODUCTION

THE uterus is bicornuate and the two cornua are morphologically bilaterally symmetrical in all the bats except in the members belonging to the family Phyllostomatidae which have simplex uterus. However, from a physiological point of view the bats possessing bicornuate uterus can be recognized into three major categories: (1) Those in which ovulation normally occurs from both the ovaries and pregnancy is carried in both the uterine cornua as in several vespertilionids[1-8]. (2) Those in which only one ovary normally releases a single ovum during each cycle and the embryo is carried in the ipsilateral uterine cornu, but the two sides of the genitalia alternate in releasing the ovum and in carrying pregnancy in successive cycles as in *Desmodus rotundus murinus*[9], *Taphozous longimanus*[10,11] and *Rousettus leschenaulti*[12,13]. (3) Those in which, although the two sides of the genitalia are morphologically symmetrical, there is a distinct physiological dominance of one side in releasing the ovum and in carrying the pregnancy. Three different conditions can further be recognized among the bats of the third category—(*a*) those where only the right ovary is functional and pregnancy invariably occurs in the right uterine cornu as in the British horse shoe bats[14], and a few South African bats[15]; (*b*) those where the left ovary is invariably functional except in rare cases where the right ovary may also release an ovum in addition to the left ovary as in *Megaderma lyra lyra*[16-20]; (*c*) those where the left side of the genitalia is functional in about 70% of the cases and the right in about 30% as in *Hipposideros fulvus fulvus*[21] and *H. ater ater*[22].

MATERIAL

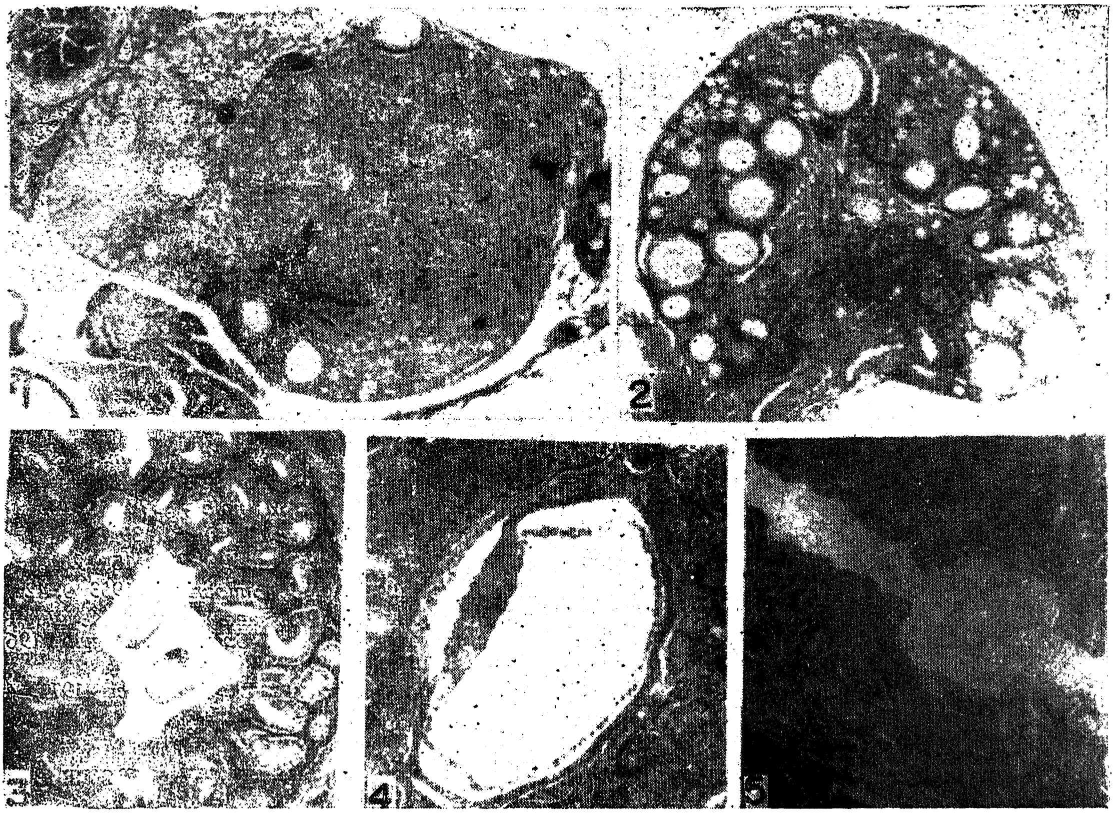
Although *Rhinolophus rouxi* breeds in a sharply defined season and the female produces a single young one each time[23,24] this bat exhibits certain special reproductive adaptations to suit different ecological conditions so that the young ones are delivered during the most propitious period when the mother in lactation and the weaned young ones get abundant food supply, namely the insects[25]. During the course of a detailed study of the breeding behaviour of this species, the present authors noted that among 285 pregnant specimens examined, 283 females had undergone ovulation in the right ovary and carried the embryo in the right uterine cornu, while the remaining two exceptional cases exhibited unique characters which are of considerable significance in view of the pronounced physiological asymmetry, with dextral dominance of the female genitalia. Since these two cases pose interesting problems concerning the physiology of reproduction in particular and of other monotocous bats with unilateral dominance of the female genitalia in general, it was felt that the description of these two unique cases would be of considerable theoretical interest.

OBSERVATIONS AND DISCUSSION

In one of the two exceptional cases the right ovary had released a single ovum as indicated by the fact that the ovary contained a single large corpus luteum (Fig. 1). The left ovary had not ovulated but contained numerous follicles at various stages of development or undergoing atresia (Fig. 2). However, there were two free unilaminar blastocysts in the right uterine cornu (Fig. 3). The two blastocysts were of the same size, and contained nearly the same number of cells in the inner cell mass. The two blastocysts were lying in the uterine lumen at the same level and extended through the same number of transversely cut series of sections of the uterine cornu. The two blastocysts were completely independent of each other and each blastocyst had its own covering of the zona pellucida. The fact that there was a single corpus luteum in the right ovary and none in the left indicates that the two blastocysts were monozygotic twins which had become separated either during the first cleavage of the egg or at some stage during the early development of the embryo.

Only one unquestionable case of a monozygotic twins in a bat has been described so far in the case of *Pipistrellus ceylonicus chrysothrix*[26] in which the twin embryos were synchorial and these had undergone implantation and were in late neural groove stage of development. Karim[27] described a rare case of synchorial twins in *Hipposideros fulvus fulvus*, but she could not determine if they were monozygotic. Similarly, exceptional cases of twinning have been mentioned in a few more bats which are normally monotocous[19,20,28-33]. But none of these has been shown to be unquestionably monozygotic.

The second anomalous case noticed during the present investigations refers to a specimen in which both ovaries had undergone ovulation as revealed by the fact that a corpus luteum was present in each ovary. The right uterine cornu contained a late implanted blastocyst (Fig. 4) and the left cornu had a free bilaminar blastocyst (Fig. 5). Evidently the blastocyst in the right cornu was more advanced in development than the one in the left cornu. Apart from the fact that the occurrence of ovulation from both the ovaries in this species is in itself unique, the difference in the stage of development of the two blastocysts requires explanation. There are only two other reports of analogous cases in the bats, and both the reports refer to *Megaderma lyra lyra*[18,19]. In both these cases the embryo in the left uterine cornu (which normally carries the conceptus in this species) is far more advanced in development than the one in the right cornu. Whereas no explanation was given for the differential development of the two embryos in *Megaderma lyra lyra* since the embryos studied were far advanced in development and since there was no other evidence which could be used for giving an explanation, *Rhinolophus rouxi* offers evidence for a plausible explanation for this phenomenon. The size and histology of the corpus luteum are identical in the two ovaries thereby indicating that ovulation must have occurred simultaneously in the two ovaries. The left side of the genitalia in this species does not normally



FIGS. 1–5. Fig. 1. Right ovary of the exceptional specimen with twin blastocysts. Note the large corpus luteum. Fig. 2. Left ovary of the same specimen as above. See text for descriptions. Fig. 3. Part of the section of the uterus containing the twin blastocysts. Note the complete independence of the two blastocysts. This is the right uterine cornu. Fig. 4. Section of the right uterine cornu of the second exceptional specimen. Note the late implanted blastocyst. Fig. 5. Section of the left uterine cornu of the specimen mentioned in Fig. 4. Note the free blastocyst in the uterine lumen. (All figures are photomicrographs and magnified to the same mangification. × 96.)

exhibit progestational reaction during normal cycles when only the right ovary releases an ovum and develops a corpus luteum. There is, perhaps, a special mechanism in this bat as in a few other bats[34-36] in which the corpus luteum induces progestational reaction only in the ipsilateral uterine cornu by short-circuiting the flow of progesterone directly into the ipsilateral uterine cornu through a portal system between the ovary and the corresponding uterine cornu. However, in the exceptional specimen in which both the ovaries ovulate and develop a corpus luteum each, the progestational reaction in the left cornu is less vigorous and slower than in the right cornu. Consequently, the environment in the left cornu does not provide as efficient a hormonal and nutritional level as that in the right cornu. Hence, the preimplantation development of the embryo in the left cornu is retarded and the blastocyst in the abnormal cornu remains unimplanted for some time while the one in the normal cornu undergoes implantation and further development. After the difference in the development of the two embryos is established, this difference is maintained until parturition. Consequently, the two embryos are always different in size and in the degree of development at any given stage of gestation. This explanation would be valid also for the two abnormal cases of *Megaderma lyra lyra*[18,19], where both the uterine cornua carried an embryo each, and where the embryo in the normal cornu was larger and was more advanced in development than the one in the abnormal cornu.

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1. Lyon, M. W., *Proc. U.S. Nat. Hist. Mus.*, 1903, **20**, 425.
2. Ramaswami, L. S., *J. Mys. Univ.*, 1933, **7**, 1.
3. Wimsatt, W. A., *J. Mammal.*, 1945, **26**, 23.
4. Gopalakrishna, A., *Proc. Ind. Acad. Sci.*, 1947, **26**, 219.
5. Uchida, T., *Sci. Bull. Fac. Agr., Kyushu Univ.*, 1950, **12**, 11.
6. McKean, J. L. and Hamilton-Smith, E., *Victoria Natur.*, 1967, **84**, 203.
7. Madhavan, A., *Mammalia*, 1971, **35**, 283.
8. Gopalakrishna, A., Thakur, R. S. and Madhavan, A., *Dr. B. S. Chauhan Comm. Vol.*, 1975, p. 225.
9. Wimsatt, W. A. and Trapido, H. T., *Amer. J. Anat.*, 1952, **91**, 415.
10. Gopalakrishna, A., *Curr. Sci.*, 1954, **23**, 60.
11. —, *Proc. Nat. Inst. Sci. India*, 1955, **21**, 29.
12. —, *Curr. Sci.*, 1964, **33**, 558.
13. — and Choudhari, P. N., *J. Bombay Nat. Hist. Soc.*, 1977, **74**, 1.
14. Matthews, L. H., *Trans. Zool. Soc. London*, 1937, **23**, 224.
15. —, *Proc. Zool. Soc. London*, 1941, **111**, 289.
16. Gopalakrishna, A., *Proc. Nat. Inst. Sci. India*, 1950, **16**, 93.
17. Ramakrishna, P. A., *J. Mys. Univ.*, 1951, **12**, 107.
18. Ramaswamy, K. R., *Proc. Nat. Inst. Sci. India*, 1961, **27**, 287.
19. Ramaswami, L. S. and Anandkumar, T. C., *Experientia*, 1963, **19**, 321.
20. Gopalakrishna, A., Khaparde, M. S. and Sapkal, V. M., *J. Bombay Nat. Hist. Soc.*, (In press).
21. Madhavan, A., Patil, D. R. and Gopalakrishna, A., *Ibid.*, (In press).
22. Gopalakrishna, A. and Madhavan, A., *Ibid.*, (In press).
23. Brosset, A., *Ibid.*, 1962, **59**, 707.
24. Gopalakrishna, A. and Rao, K. V. B., *Ibid.*, (In press).
25. Ramakrishna, P. A. and Rao, K. V. B., *Curr. Sci.*, 1977, **46**, 270.
26. Gopalakrishna, A. and Phansalkar, R. B., *Ibid.*, 1970, **39**, 309.
27. Karim, K. B., *Ibid.*, 1973, **42**, 823.
28. Moghe, M. A., *Bull. Zool. Soc. Coll. Sci. Nagpur*, 1960, **2**, 1.
29. Bradshaw, G. V. R., *Science, N.Y.*, 1962, **135**, 645.
30. Barlow, J. and Tamsitt, J. R., *Can. J. Zool.*, 1968, **46**, 290.
31. Davis, W. B., *J. Mammal.*, 1970, **51**, 105.
32. Burns, R. J., *Ibid.*, 1970, **51**, 391.
33. Medway, Lord, *J. Zool.*, 1971, **165**, 261.
34. Marshall, A. J., *J. Endocrinol.*, 1953, **9**, 42.
35. Gopalakrishna, A. and Murthy, K. V. R., *Bull. Zool. Soc. Coll. Sci. Nagpur*, 1960, **2**, 19.
36. — and Karim, K. B., *Curr. Sci.*, 1971, **40**, 490.

COBALT TOXICITY AND ITS REVERSAL BY IRON AND MAGNESIUM IN *OGAWA* SEROTYPES OF *VIBRIO CHOLERAE* AND *VIBRIO ELTOR*

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ABSTRACT

Toxic effects of Co^{2+} on growth, glucose utilisation and acid production were studied, and the capability of Fe^{3+} and Mg^{2+} for counteracting Co^{2+} toxicity was investigated in *Ogawa* serotypes of *Vibrio cholerae* and *Vibrio eltor*. Bivalent Co induced 50% growth inhibition in *V. cholerae Ogawa* and *V. eltor Ogawa* at 150 μg and 100 μg per 10 ml test medium respectively. It was toxic to both the biotypes and destroyed their metabolic activity at 250 μg. Co^{2+} toxicity was reversed by both Fe^{3+} and Mg^{2+} to a varying degree in *V. eltor Ogawa*. In *V. cholerae Ogawa*, Mg^{2+} reversed Co^{2+} effects to a considerable extent while Fe^{3+} supplementation caused the total extinction of all the metabolic parameters in Co^{2+} toxicosed cells. The results indicate interesting interactions between Co^{2+}, Mg^{2+} and Fe^{3+} in these strains.

INTRODUCTION

In compound and ionic form, elements such as H, Na, K, Mg, Ca, Fe, C, N, P, O and S are constituents of many types of living cells, whereas several other elements occur in smaller amounts or as trace elements[1]. Metals of low atomic weights are less toxic than those of high atomic weight; moreover, bivalent metals are more toxic than monovalent metals[2].

Extra physiological concentrations of certain heavy metals are known to produce pathological changes in animals, plants and micro-organisms. The toxic effects of different concentrations of Co, Zn, Ni and Mo in mostly non-pathogenic organisms have been demonstrated. Responses towards certain pairs of metals varied and the interaction occurring in one microbial species need not occur in others. In *Neurospora crassa* deranged Fe and Mg metabolism particularly caused by Co was observed[3]. In *N. crassa* an Fe-binding compound has been isolated from the culture fluid of organisms grown under conditions of Co-toxicity[4]. Further, increasing concentrations of Co in the medium resulted in the increased production of an Fe-binding compound and a corresponding fall in the catalase activity of *N. crassa*[5]. Co-toxicity was correlated with Fe deficiency in *Aspergillus niger*[6] and in *Micrococcus pyogenes* var. *aureus*[7], Fe was more effective than Mg in the alleviation of Co-toxicity in these organisms. Cobalt-inhibited growth of *A. niger* was accompanied by decreased glucose utilisation and acid production and under these conditions the metabolism of several organic acid intermediates of glucose breakdown was affected[8].

The current report is concerned with the study of interrelationships between Co^{2+} toxicity and its reversal by Fe^{3} and Mg^{2+} in *Ogawa* serotypes of pathogenic vibrios.

MATERIALS AND METHODS

V. cholerae neotype strain, *Ogawa* serotype **(NCTC 8021) and *V. cholerae*** biotype *eltor, Ogawa* serotype (NCTC 10255) obtained from Central Public Health Laboratory, London, were used.

Cobalt chloride (BDH), ferric ammonium citrate (Merck) and magnesium sulphate (BDH) were of Analar grade. Ferric ammonium citrate was used in the present study since ferrous salts are easily oxidised to ferric form. Sterile glass-distilled water was employed in preparing metal solutions separately so that 100 ml of each solution contained 100 mg of the metal. The final concentrations were expressed as μg of metal per tube containing 10 ml test medium.

Each 100 ml lot of test medium contained peptone (1 g), sodium chloride (0·5 g), and glucose (1 g). Each 100 ml lot of peptone water contained only peptone, (1 g), and sodium chloride (0·5 g). The pH of these culture media was adjusted to 7·6.

Cobalt solution was added aseptically to the tubes containing 10 ml test medium to provide the metal concentrations ranging from 50–1000 μg/tube. Experiments conducted to determine toxicity reversals contained Co^{2+} sufficient to induce 50% growth inhibition and reversing metals, Fe^{3+}/Mg^{2+} in concentrations from 50–1000 μg per tube. Aliquots of 0·05 ml of 20 h peptone water culture of vibrios containing approximately 10^8 viable cells were used to inoculate the tubes. After incubation for 24 h, the growth was measured colorimetrically at 660 nm. Glucose was estimated by the technique of Follin and Wu[9]. Acid production was determined by titrating 2 ml aliquots of culture medium against 0·005 N NaOH using bromothymol blue as indicator. All experiments were repeated 5 times and the average values were taken.

RESULTS

Co^{2+} effects on growth. Bivalent Co was toxic to both the biotypes (Table I). It induced 50% growth inhibition in *V. cholerae Ogawa* and *V. eltor Ogawa* at 150 μg and 100 μg respectively. While the growth of these pathogenic vibrios was almost at par with 50 μg, it reached extinction level at 250 μg in both the biotypes. At 200 μg, the growth was inhibited by 90% in *V. cholerae* and 68% in *V. eltor.* Co^{2+} thus appears less tolerated by *V. cholerae Ogawa* than by *V. eltor Ogawa* beyond 150 μg although the tolerance of the former to Co^{2+} at 100 μg was greater than that of the latter, the growth values being 70% and 50% respectively.

Co^{2} effects on glucose utilisation and acid production. At concentration of Co^{2+} inducing 50% growth inhibition, glucose utilisation and acid production values reached 50% and 45% of maximals respectively in *V. cholerae Ogawa,* and 65% and 75% in *V. eltor Ogawa* (Table I). Interestingly, at 200 μg, *V. cholerae Ogawa* achieving a growth of only 10% removed 33% of the initial supply of glucose without any acid production. This suggests the possible **suppression** of catabolic activity at this metal concentration, although glucose was imbibed by the Co^{2+} **treated** cells. In contrast, in *V. eltor Ogawa* yielding a growth of 32% at 200 μg, the utilisation and production values were 48% and 50% respectively. Further, at and above 200 μg, Co^{2+} **totally** destroyed the metabolic activity in both the biotypes as evident from the values of all the responses reaching extinction levels.

Effects of Fe^{3+} and Mg^{2+} on Co^{2+} ***toxicity.***— In *V. cholerae Ogawa,* Fe^{3}, besides its absolute failure to alleviate Co^{2} toxicity, also caused total extinction of all metabolic responses, while Mg^{2} reversed Co^{2} influences to a considerable degree (Table II). On the other hand, both Fe^{3} and Mg^{2+} reversed Co^{2+} effects in *V. eltor Ogawa* to a varying extent (Table III).

It is evident from Table II that supplementation of Fe^{3+} from 50 to 1000 μg did not restore Co^{2} inhibited bacterial growth, glucose utilisation and acid production to any degree in *V. cholerae Ogawa.* Interestingly, Fe^{3} supplementation resulted in the destruction of metabolic functions as revealed by the values of all the responses reaching extinction levels. Experiments to determine the effects of Fe^{3+} alone on these vibrio types revealed 100% growth, glucose utilisation and acid production.

TABLE I

Cobalt^{2+} effects on metabolic parameters in Ogawa *serotype of* V. cholerae *and* V. eltor*

	Co^{2+} in μg/10 ml medium						
	0	50	100	150	200	250	300
			Ogawa serotype of *V. cholerae*				
Growth	100	74	70	50	10	0	
Glucose utilisation	100	100	75	50	33	0	
Acid production	100	84	73	45	0	0	
			Ogawa serotype of *V. eltor*				
Growth	100	72	50	42	32	0	
Glucose utilisation	100	82	65	62	48	0	
Acid production	100	81	75	68	50	0	

* Values expressed as percentage of control.

TABLE II

Influences of Fe^{3+} and Mg^{2+} supplementation on Co^{2+} effects in Ogawa *serotype of* V. cholerae*

Analysis	Fe^{3+}/Mg^{2+} in μg/10 ml medium													
	c_1	c_2	50	100	150	200	250	300	400	500	600	700	800	900
							$Co^{2+} + Fe^{3+}$							
Growth	100	50	0	0	0	0	0	0	0	0	0	0	0	0
Glucose utilisation	100	50	0	0	0	0	0	0	0	0	0	0	0	0
Acid production	100	45	0	0	0	0	0	0	0	0	0	0	0	0
							$Co^{2+} + Mg^{2+}$							
Growth	100	50	54	65	72	72	72	72	72	72	72	72	72	72
Glucose utilisation	100	50	80	80	100	100	100	100	100	100	100	100	100	100
Acid production	100	45	60	60	65	70	70	74	74	70	70	64	60	60

* Values expressed as percentage of control.
c_1 Control without any metal.
c_2 Control with Co^{2+} (150 μg) inducing 50% growth inhibition.

In contrast, 150 μg of Mg^{2} restored bacterial growth of 72% with 100% glucose utilisation. Increasing concentrations of this metal above the maxima did not improve the growth pattern which remained at the same level and the situation of glucose utilisation also unchanged remaining 100%. Supplementation with 50–400 μg of Mg^{2+} restored acid production from 60% to 74% with a gradual slight fall at higher concentrations.

Table III shows that in *V. eltor Ogawa,* 200 μg of Mg^{2} restored maximal growth of 85%. Addition of 50–500 μg of Fe^{3+} caused only a slight improvement in growth (10%) and acid production while glucose utilisation level remained the Interestingly, higher concentrations of Fe^{3} inhibitory to all the three parameters tested. the other hand, supplementation of 50–1000 μg Mg^{2+} restored bacterial growth from 50% to glucose utilisation from 65% to 87% and production from 75% to 81%.

DISCUSSION

From the data presented here, Mg^{2+} is effective than Fe^{3} in the alleviation of

TABLE III

Influences of Fe^{3+} and Mg^{2+} supplementation on Co^{2+} effects in Ogawa *serotype of* V. eltor*

Analysis	Fe^{3+}/Mg^{2+} in μg/10 ml medium														
	c_1	c_2	50	100	150	200	250	300	400	500	600	700	800	900	1000
	$Co^{2+} + Fe^{3+}$														
Growth	100	50	55	60	60	60	60	60	60	60	50	45	34	34	34
Glucose utilisation	100	65	65	65	65	65	65	65	65	65	60	52	52	52	52
Acid production	100	75	79	80	81	81	81	81	81	81	79	75	66	66	66
	$Co^{2+} + Mg^{2+}$														
Growth	100	50	66	76	81	85	85	85	85	85	85	85	85	85	85
Glucose utilisation	100	65	65	65	87	87	87	87	87	87	87	87	87	87	87
Acid production	100	75	75	77	81	81	81	81	81	81	81	81	81	81	81

* Values expressed as percentage of control.

c_1 Control without any metal.

c_2 Control with Co^{2+} (100 μg) inducing 50% growth inhibition.

deleterious effects of Co^{2+} in *V. eltor Ogawa.* While beneficial influences are seen with Mg^{2+} in *V. cholerae Ogawa,* Fe^{3+} not only has failed in ameliorating Co^{2+} effects but also caused absolute toxicity resulting in total extinction of all metabolic responses. The salient differences noticed in the specific reversal pattern of Co^{2+} toxicity are contrary to the observations reported for *M. pyogenes* var. *aureus*[7] and by Adiga *et al.*[6], for *A. niger* where Fe^{3+} was more effective than Mg^{2+} in reversing Co^{2+} toxicity.

Further, Fe^{3+} alone is not toxic to *V. cholerae Ogawa.* This indicates that Fe^{3+} as such has no adverse effects on this vibrio to any extent, while it is drastically toxic in combination with Co^{2+}. On the other hand, it is interesting to recall our previous observation that although Fe^{3+} did not enhance Ni^{2+} toxicity, it totally failed to alleviate this effect in *V. cholerae Ogawa*[10]. The exact mechanisms in which Fe^{3+} functions as totally toxic to Co^{2+} treated cells of *V. cholerae Ogawa* in their metabolism remains to be investigated. This observation may indicate biochemical differences between the two biotypes, and more detailed enzyme studies may reveal the intricate mechanism involved in their metabolic functions.

The difference observed in metabolic responses when the cells are exposed to Co^{2+} at 200 μg in *V. cholerae Ogawa* and *V. eltor Ogawa* appear to be significant since it was consistent. Also the reversal findings with Fe^{3+} indicate marked differences in the pattern of behaviour of the two strains tested.

ACKNOWLEDGEMENT

We thank Dr. S. Lapage, Curator, Central Public Health Laboratory, London, for kindly supplying the bacterial strains.

1. Frobisher, M., *Fundamentals of Microbiology,* 8th ed., W. B. Saunders Co. (Philadelphia), 1968, p. 70.
2. Wilson, G. S. and Miles, A. A., *Topley and Wilson's Principles of Bacteriology and Immunity,* Edward Arnolds Ltd. (London), 1964, p. 141.
3. Sivarama Sastry, K., Adiga, P. R., Venkatasubramanyam, V. and Sarma, P. S., *Biochem. J.,* 1962, 85, 486.
4. Padmanabhan, G. and Sarma, P. S., *Ibid.,* 1964, 98, 330.
5. — and —, *Arch. Biochem. Biophys.,* 1965, 111, 147.
6. Adiga, P. R., Sivarama Sastry, K., Venkatasubramanyam, V. and Sarma, P. S., *Biochem. J.,* 1961, 81, 545.
7. Narasimha Rao, S. and Nagesha, C. N., *Indian J. Expt. Biol.,* 1967, 5, 182.
8. Sivarama Sastry, K., Adiga, P. R., Padmanabhan, G. and Sarma, P. S., *Arch. Biochem. Biophys.,* 1962, 99, 351.
9. Folin, O. and Wu, H., *J. Biol. Chem.,* 1920, 41, 367.
10. Lalithamma, B. P., Karuna Sagar, I., Narasimha Rao, S. and Nagesha, C. N., *Curr. Sci.,* 1976, 45, 578.

LETTERS TO THE EDITOR

MIXED-VALENT PALLADIUM (IV AND II) COMPLEXES OF TETRADENTATE SCHIFF BASES DERIVED FROM ACETYLACETONE AND DIAMINES—NOVEL EXAMPLES OF PALLADIUM(IV)-OXO-π-ALLYLIC BONDING

Introduction

THE formation of π-allylic complexes is now recognized as a general feature of transition metals and several reviews on this subject have appeared in the literature[1-5]. Of all the metal ions palladium in its lower oxidation states, 0 and + 2, are known to form maximum number of π-allylic complexes. However, there seems to be no report on similar type of bonding involving higher oxidation states of palladium. This communication reports the first examples of mixed-valent palladium complexes involving Pd(IV)-Oxo-π-allylic bonding with bivalent tetradentate Schiff bases derived from acetylacetone and diamines.

Experimental

Materials and Physical Measurements: N, N′-diamine-bis (acetyl-acetoneimine)[6] and K_2PdCl_4 (Ref. 7) were prepared by the reported methods. $PdCl_2$ (Johnson–Mathey, Ltd.) was used as such. Organic solvents were of analar grade. The physico-chemical measurements of the complexes were made as described before[8].

Preparation of the Complexes: The complexes $[Pd^{IV} L] [Pd^{II} Cl_4]$, were prepared by adding ice-cold aqueous solution of an appropriate N, N′-diamine-bis (acetylacetoneimine) to an excess of $PdCl_2$ or K_2PdCl_4 in water which is also previously cooled to 0° C. The orange-red crystals obtained were collected by filtration, washed with water and recrystallized from a large volume of chloroform. The yields of the complexes were found to be temperature dependent, being maximum at 0° C (50–60%) and decreasing with increase in temperature. The formation of these complexes is very specific to the aqueous medium as well as the sequence of addition of the reagents. The latter condition is very critical since the addition of $PdCl_2$ or K_2PdCl_4 to the excess of ligand solution yielded yellow crystalline compounds, which were found to be identical with the already reported compounds[6], having structure (B).

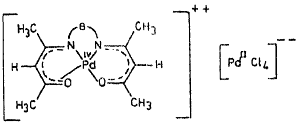
Results and Discussion

The orange red complexes gave satisfactory elemental analyses corresponding to the molecular formula $[Pd^{IV} L]$ $[Pd^{II} Cl_4]$. The complexes in contact with acetonitrile for several hours deposit metallic palladium as a silver mirror. The reduction of the complexes to metallic palladium was found to be instantaneous in presence of hydrazine hydrochloride. The electrical conductivity of the complexes in acetone and acetonitrile (concentration $\sim 10^{-3}$ M) shows that they are 1 : 1 electrolytes ($\Lambda_M < 120 - 160$ Ohm^{-1} cm^2 mole^{-1}). The diamagnetic nature of the complexes is indicative of square-planar geometry around Pd^{IV} and Pd^{II}.

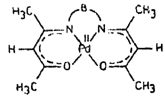
The electronic absorption spectra of the complexes in nujol mull show four bands in the ultraviolet region. The broad band centered at 39,700 cm^{-1} is due to the ligand transitions of $[Pd^{IV} L]^{2+}$, while the three bands occurring around 43,000, 30,000 and 22,000 cm^{-1} are characteristic of $PdCl_4^{2-}$ ion[9]. The presence of $PdCl_4^{2-}$ ion indirectly supports that the oxidation state of palladium in the cation is IV. This is further supported by the fact that the complexes liberate iodine from an acidified solution of KI. Although these results confirm the presence of Pd(IV) in the complexes, it is not clear how a portion of Pd(II) is oxidised to Pd (IV). It is probable that under the experimental conditions employed, water oxidises Pd(II) to Pd(IV), as even in an inert atmosphere the same product is obtained.

The ir spectra of the complexes in nujol mull show bands due to π-CH, δ-CH and coordinated νCN, respectively around 760, 1190 and 1575 cm^{-1}. Two intense bands are observed around 1520 and 1630-1650 cm^{-1} region. The former band is due to coordinated νCO. The occurrence of the latter indicates that the second carbonyl group of the coordinated ligand is not conventionally bonded through oxygen. However, the position of this band is too low for it to be a non-coordinated acetyl carbonyl, which generally absorbs around 1700 cm^{-1} (Ref. 10). Their data could be accounted for by postulating Pd^{IV}-oxo-π-allylic bonding as shown in structure A. Bonding of this type has been assigned to di-μ-chloro-bis (2-phenyl-3-chloro-π-oxapropenyl) dipalladium(II)[11]. The possibility of Pd^{IV}-γ-carbon bonding is ruled out since the model building shows considerable strain for such structures.

The complexes readily react with π-acids like triphenyl-phosphine and triphenylarsine, giving diamagnetic penta-coordinated adducts. These complexes show the absence of the band around 1630 cm^{-1} indicating the conventional coordination of the carbonyl oxygen to Pd^{IV}. Hence, in presence of π-acids the oxo-π-allylic bond is weakened and the latter coordination is favoured. The results of these studies along with reactions of A with primary amines and nitrosating reagents will be published elsewhere.



A



B

(I) B : $-CH_2CH_2-$
(II) B : $-CH_2CH(CH_3)$
(III) B : $-CH_2CH_2CH_2-$

Although four methyl proton signals are expected for the structure of the type A, the pmr spectrum of A(I) in $CDCl_3$ shows only three signals at 8·42, 8·00 and 7·94 τ with integrated intensities of 6:3:3, which are assignable respectively to $[-(CH_3)C(=N)-]$, $COCH_3$ (Oxygen-bonded ring) and $COCH_3$ (π-allylic bonded ring). The merging of the methyl proton signals in the vicinity of the ethylene skeleton may be attributed to their identical shielding parameters. The γ-CH proton gives a broad singlet at 5·12 τ. The complex multiplet centered at 6·52 τ is due to ethylene protons. The pmr spectrum of A(II) shows spectral features analogous to that of A(I).

In conclusion, at present, $[Pd^{IV} L]$ $[PdCl_4]$ are the only known mixed-valent palladium complexes, in which Pd^{IV} shows oxo-π-allylic bonding.

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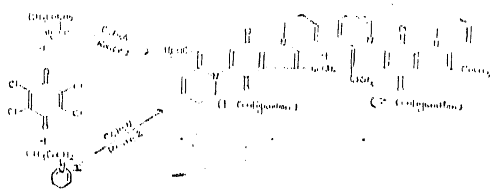
1. Guy, R. G. and Shaw, B. L., *Advan. Inorg. Chem. Radiochem.*, 1962, **4**, 78.
2. Green, M. L. H. and Nagy, P. L. I., *Advan. Organometal. Chem.*, 1964, **2**, 325.
3. —, *Organometallic Compounds*, Methuen, London, 1968, **2**, 39.
4. Wilke, G., Bogdanovic, B., Hardt, P., Heimbach, P. Keim, W., Kroner, M., Oberkirch, W., Tanaka, K., Steinrucke, E., Walter, D. and Zimmermann, H., *Angew. Chem. Int. Ed. Engl.*, 1966, **5**, 151.
5. Lobach, M. I., Babitskii, B. D. and Kormer, V. A., *Russ. Chem. Rev.*, 1967, p. 476.
6. Ueno, K. and Martell, A. E., *J. Phys. Chem.*, 1955, **59**, 998.
7. Mizuna, J., *J. Pharm. Soc. Japan*, 1949, **69**, 126.
8. Iyengar, R. R., Bose, K. S. and Patel, C. C., *Inorg. Chim. Acta*, 1973, **7**, 3.
9. Cohen, A. J. and Davidson, N., *J. Am. Chem. Soc.*, 1955, **73**, 1951.
10. Nakamoto, K., in *Infrared Spectra of Inorganic and Coordination Compounds*, 2nd Ed., Wiley-Interscience, New York, 1970.
11. Yoshimura, N., Murahashi, S. T. and Moritani, I., *J. Organometal. Chem.*, 1973, **52**, C58.

A NEW PROCEDURE FOR THE SYNTHESIS OF SUBSTITUTED PYRROCOLINE QUINONES

Introduction

The synthesis of a number of 5:11 disubstituted and 5:7 disubstituted pyrrocoline quinones has been reported by Tilak *et al.*[1]; by the oxidative condensation of chloranil with pyridine and active methylene compounds, like acetyl acetone, acetoacetic ester or malonic ester. That both malonic ester and acetoacetic ester gave the same products was explained on the basis that in the final stage the more electron withdrawing group was cleaved.

It was therefore thought logical to use some other compounds like acetonyl pyridinium iodide (CH_3–CO–CH_2–$N^+C_5H_5I^-$) and its analogues in which an active methylene group is situated between a carbonyl group and a strongly electron withdrawing $>N^+-$ (quaternary nitrogen). It should be the strongly electron withdrawing $>N^+-$ group which should be cleaved at the final stage. The resulting product in this particular case should be identical with the product obtained by the condensation of chloranil with acetyl acetone and pyridine as shown in the scheme below :



It may be seen that the use of acetonyl pyridinium iodide or acetonyl quinolinium iodide should give the same products and it is actually observed that the products of reaction (i) and (ii) are the same as verified through T.L.C. The nature of Z and E isomers of pyrrocoline quinones is ascertained through their characteristic colour reaction with sulphuric acid. In reaction (ii), if we take quinoline or isoquinoline instead of pyridine as the base, only E configuration benzo dibenz pyrrocoline quinone is obtained and is found to be identical with the compounds reported earlier[2]. The formation of compounds of Z configuration seems to be sterically hindered. The identity of the compounds prepared by both the routes was ascertained through T.L.C.

The use of the various substituted keto alkyl quaternary ammonium salts $(R{-}CO{-}CH_2{-}N{-}\underset{I^-}{N}\langle\ )$ should give different substituted derivatives of pyrrocoline quinones. This should be a general reaction for preparing such compounds. Further work in synthesising these compounds is in progress.

Experimental

All melting points have been determined on a Kofler instrument and are uncorrected.

N-Acetonyl pyridinium/quinolinium iodide.—These compounds are prepared and purified by the known methods[3].

Benzo-dibenz (h, h′)-pyrrocoline quinones—The following general procedure was adopted:—A mixture of chloranil (1 mole), N-acetonyl pyridinium iodide or N-acetonyl quinolinium iodide (1 mole) and quinoline (1 mole) in 25–30 ml ethanol was heated under reflux on a water bath for 4 hours, when all the chloranil dissolved and a deep red coloured reaction mixture was obtained. The product separated on cooling was filtered and washed with ethanol, ether, hot water and then dried. The product was recrystallised twice from pyridine. Benzo-dipyrrocoline quinones. Same procedure was adopted while using N-acetonyl pyridinium iodide, chloranil and pyridine. The E and Z products were separated through chromatography over alumina using benzene and methanol as eluants.

Compounds :

(1) 7 : 15-diacetyl-benzo (1 : 2-*b*, 4 : 5-*b′*)-dibenz (*h*, *h′*) pyrrocoline quinone—m.p. does not melt upto 356° (Found : C, 76·5; H, 3·8; N, 5·9; $C_{30}H_{18}N_2O_4$ requires C, 76·5 ; H, 3·8, N, 5·9%).

(2) 5 : 11-diacetyl-benzo (1 : 2-*b*, 4 : 5-*b′*)-dipyrrocoline quinone (E form)—m.p. does not melt upto 356° (Found : C, 71·1; H, 3·5 ; N, 7·4 ; $C_{22}H_{14}N_2O_4$ requires C, 71·3; H, 3·7; N, 7·5%).

(3) 5 : 11-diacetyl benzo (1 : 2-*b*, 4 : 5-*b′*)-dipyrrocoline quinone (Z form)—m.p. does not melt upto 356° (Found: C, 71·2 ; H, 3·4 ; N, 7·3 ; $C_{22}H_{14}N_2O_4$ requires: C, 71·3; H, 3·7 ; N, 7·5%).

Thanks are due to Professor R. C. Kapoor, Head of Chemistry Department, for providing necessary Laboratory facilities and to the U.G.C., New Delhi, for the award of a Junior Research Fellowship to R. P. S.

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1. Tilak, B. D. and Venkiteswaran, M. R., *J. Sci. Indi. Res.* (*India*), 1956, **15 B**, 561.
2. Soni, R. P. and Saxena, J. P., *J. Indian Chem. Soc.* (Communicated).
3. Saxena, J. P. and Galera, M. R., *Aust. J. Chem.*, 1967, **20**, 1771.

CHEMICAL EXAMINATION OF THE LEAVES OF *GUAZUMA TOMENTOSA* KUNTH

The presence of friedelin 3β-ol, friedelin 3α-acetate, β-sitosterol besides a plant alcohol is reported from the leaves of *Guazuma tomentosa* Kunth.

Guazuma tomentosa Kunth (N.O. Sterculeaceae) is a moderate sized deciduous tree indigeneous to tropical America and introduced into India[1]. The presence of kaempferol, quercetrin and kaempferitrin were reported earlier from the flowers of *G. tomentosa* Kunth[2]. Isolation and characterisation of friedelin, β-sitosterol and betulin have been recently reported by us from the hexane extract of its bark[3]. Chemical examination of the leaves is now reported.

The air dried leaves (1·2 kg) were extracted with petroleum ether. The extract after concentration left a residue which resisted crystallisation. It was chromatographed over a column of silica gel. The first three fractions (500 ml) of the hexane eluate gave only waxes. The next two fractions gave Compound A (0·08 m). Further elution with hexane: benzene (9 :1) gave Compound B (0·1 gm), with hexane-benzene (3 : 1) Compound C (0·5 gm), with hexane : benzene (7 : 3) Compound D (1·0 gm) and further elution with hexane : benzene (1 : 1) and benzene did not yield any crystalline compounds.

Compound A, $C_{32}H_{54}O_2$, crystallised from petroleum ether-chloroform as colourless shining needles, m.p. 306–8°, $[\alpha]_D^{30^\circ} - 14^\circ$. It gave Liebermann Burchard test positive for triterpenes. It indicated acetoxyl group (1730 and 1250 cm^{-1}) in I.R. Compound A on hydrolysis with 5% methanolic KOH furnished a compound, $C_{30}H_{50}O$, m.p. 300–301° which was identified as friedelin 3α-ol by direct comparison (m.m.p. and I.R.) with an authentic sample. Compound A is thus friedelin 3α-acetate.

Compound B, $C_{30}H_{50}O$, crystallised from chloroform-petroleum ether as colourless needles, m.p. 278–279°, $[\alpha]_D^{30^\circ} + 14^\circ$. It answered positive LB test for triterpenes and gave an acetate, m.p. 288–289°. Compound B was characterised as friedelin 3β-ol by direct comparison (m.m.p. and I.R.) and through its acetate with authentic samples.

Compound C, crystallised from methanol as colourless solid, m.p. 89–90° and was found to be a plant alcohol.

Compound D, $C_{29}H_{50}O$, crystallised from chloroform-methanol as colourless needles, m.p. 137–138°, $[\alpha]_D^{30^\circ} - 36{\cdot}5$. It gave positive LB test for steroids and was identified as β-sitosterol by comparison with an authentic sample (m.m.p. and I.R.).

Our thanks are due to C.S.I.R., New Delhi, for the award of Junior Research Fellowship to one of us (V. S. M.).

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Waltair 530 003, *August* 16, 1977.

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1. Gamble, J. S., '*Flora of the Presidency of Madras*', Botanical Survey of India, Calcutta, 1967, Vol. 1, p. 79.
2. Sankara Subramanian, S. and Narayana Swamy, M., *Curr. Sci.*, 1963, **32**, 308.
3. Anjaneyulu, A. S. R. and Suryanarayana Murthy, V., Communicated to *Planta Medica*.

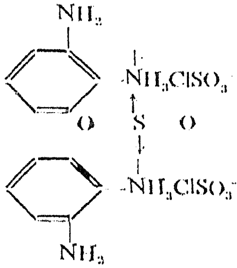
POTENTIOMETRIC TITRATIONS OF BENZENEDIAMINES IN NON-AQUEOUS MEDIUM

Benzenediamines were determined potentiometrically in methylethyl ketone by Kreshkov and Aldarova[1]. Kreshkov *et al.*[2] carried out differential titrations of mixtures of diamines in chloroform-methylethyl ketone medium. Both the groups employed perchloric acid as a titrant. The results reported by the former group were associated with an error of 2·6% and the error reported by the latter group was 3%. In the present investigation the isomeric benzene diamines have been determined potentiometrically in acetic acid-methylethyl ketone medium using chlorosulphonic acid as a titrant. The advantages of using chlorosulphonic acid in place of perchloric acid have been described earlier by Rao and Naidu[3, 4].

Chlorosulphonic acid (BDH) was used without further purification. Acetic acid (BDH) and methylethyl ketone (BDH) were purified by the method used by Rao and Naidu[3]. The three benzenediamines were purified by the method recommended by Vogel[5].

Approximately 1M stock solution of chlorosulphonic acid was prepared by adding 7 ml of the acid to 250 ml of chilled acetic acid. The solution was then diluted with methylethyl ketone to obtain the solution of required concentration. The acid solution was standardised potentiometrically employing anhydrous sodium carbonate as the primary standard. Weighed samples of bases were dissolved directly in the mixed solvent.

Titrations of bases dissolved in 1 : 1 acid-ketone medium were carried out using the Elico pH meter. The exact end point was obtained by the calculation method given in the literature[6]. Base to acid mole ratio was found to be 1 : 2 in respect of 1, 3 and 1, 4 benzenediamines and the ratio was 1 : 1·5 in the case of 1, 2 benzenediamine. These observations suggest that both the amino groups in 1, 3 and 1, 4 isomers react with the acid to form salt. In the 1, 2 isomer the acid base reaction appears to be restricted to only one amino group due to steric effect and the residual 0·5 mole of the acid may be ascribed to a condensation reaction as indicated below :



The experimental results are given in Table I

TABLE I

Base	Amount mg	
	taken	found
1, 2 benzenediamine	43·2	42·4
	21·6	21·2
1, 3 benzenediamine	43·2	42·4
	21·6	21·2
1, 4 benzenediamine	43·2	42·6
	21·6	21·2

The data in the table show that chlorosulphonic acid can be used successfully for the determination of semimicro quantities of the three diamines with an apparently negative bias and an error of ∼ 2%. The method is more accurate than that reported by Kreshkov and Aldarova[1].

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---

1. Kreshkov, A. P. and Aldarova, N. Sh., *Izv. Vysshikh Uchebn. Zavednii, Khim. Tekhnol.*, 1965, **8**, 316.
2. —, Bykova, L. N. and Pevzner, Z. D. *Zh. Obshch. Khim.*, 1965, **35**, 1332.
3. Rao, K. C. M. and Naidu, P. R., *J. Electroanal. Chem.*, 1972, **35**, 429.
4. — and —, *Talanta*, 1972, **19**, 1465.
5. Vogel, A. I., *Text Book of Practical Organic Chemistry* (Longmans), 1951, p. 612.
6. Willard, H. H. Merritt, L. L. and Dean, J. A., *Instrumental Methods of Analysis*, (Affiliated East-West Press, New Delhi), 1925, p. 552.

CHARACTERISATION OF L-DOPA IN SEEDS OF *MUCUNA SLOANEI*

L–DOPA [3-(3, 4–dihydroxyphenyl) alanine] is being used for symptomatic relief of Parkinson's disease[1]. Mucuna species AM. *puriens*, *M. andreana*, *M. mutisiana*, *M. boltoni*, *M. urens*, *M. sloanei*, *M. purvita*, etc.) are considered to be the richest natural sources of free L–dopa[2]. It is mainly concentrated in the seed embryo of these plants in concentrations of 6–9% w/w[2]. There has been no report so far on the L–dopa content of *M. sloanei* grown in Nigeria. Quickening interest in the use of L–dopa in the treatment of Parkinsonism leads us to investigate and characterise this compound in *Mucuna sloanei*, a plant which is grown widely in deciduous, secondary and Savannah regions of the world, especially Nigeria, Ghana and Sieraleon.

Experimental

Finely ground, defatted seed (50 g) was heated on a steam bath with 50 ml of 0·1N MCl for 5 minutes. After cooling, the mixture was shaken vigorously with 30 ml of 95% ethanol, and centrifuged. The supernatant was separated and an additional 50 ml of 95% ethanol was added to the residue and shaken vigorously to dissolve any L–dopa remaining. The sample was again centrifuged and the alcohol was mixed with the original supernatant and the residue discarded. The alcohol layer was subjected to T.L.C. analysis on silica gel G (0·25 mm) using solvent system propanol, ethyl acetate, water and acetic acid (20:19:10:1). A 0·1% solution of pure L–dopa in 0·1N HCl was used as a reference substance. Ninhydrin reagent was used for the detection of L–dopa on the chromatogram. A grey-blue colour was obtained for L–dopa in seed extract with hRf 0·60, a value identical with the reference L–dopa on the same plate.

Further confirmation of the identity and configuration of L–dopa was obtained by infra-red spectrum employing Perkin-Elmer instrument. IR spectrum of L–dopa isolated from the seed was in good agreement with that of pure L–dopa.

Estimation of L–dopa in seeds of *M. sloanei* was carried out by a colorimetric method described earlier[1] and was found to be 3·34% w/w dry powder. This suggests that Nigerian variety of *M. sloanei* has a low yield of L–dopa compared to the value of 6–9% for the same species grown in U.S.A.[2].

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1. Daxenbichler, M. E., VanEtten, C. H., A Hamilton, E. and Earle, F. R., *J. Med. Chem.* 1971, **14**, 463.
2. Bell, E. A. and Janzen, D. H., *Nature*, 1971, 22 136.

SYNTHESES OF 5, 5′-(*p*-PHENYLENEDIAMINE-DISAZO)-, AND 5, 5′-(*o*-DIANISIDINEDISAZO)-8-HYDROXYQUINOLINE

THE formation of a disazo dye of 8-hydroxyquinoline by coupling with benzidine is well known[1]. Here a pyridine solution of 8-hydroxyquinoline has been used for preparing disazo dyes with *p*-phenylenediamine and *o*-dianisidine. Pyridine medium is very suitable for coupling reactions[2,3].

Experimental

Preparation of 5, 5′-(*p-phenylenediaminedisazo*) and 5, 5′-(o-*dianisidinedisazo*)-8-*hydroxyquinoline*.—0·1 Mole of (i) *p*-phenylenediamine dissolved in 300 ml hot water containing 20 ml of concentrated HCl, or (ii) *o*-dianisidine dissolved in 30 ml of concentrated HCl and 400 ml of boiling water, is cooled to 5° with ice, 30 ml of concentrated HCl added, and the solution tetraazotised with 0·2 mole of a concentrated aqueous sodium nitrite solution. After a brisk effervescence of gas, the solution becomes clear and gives only a faint blue colour with starch-iodide paper. This solution is poured slowly into a solution of 0·2 mole of 8-hydroxyquinoline in 50 ml of dry pyridine and the mixture is stirred for *ca* 1 h and is kept overnight. The solid is filtered, washed with water and dried at 150°.

The purity of products was established by analyses and TLC.

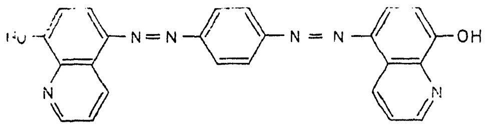
Both the compounds are dark red brown in colour, amorphous, sparingly soluble in chloroform, carbon-tetrachloride, ethanol, etc., but fairly solbuble in dimethylformamide. Melting points (uncorrected) of 5, 5′-(*o*-dianisidinedisazo)-8-hydroxyquinoline and 5, 5′-(*p*-phenylenediaminedisazo)-8-hydroxyquinoline are respectively 265° and 200° (d). These compounds behave as excellent ligands and form metal complexes which are useful for analysis.

Elemental analysis indicate the formula $C_{24}H_{18}N_6O_2$ for 5, 5′-(*p*-phenylenediaminedisazo)-8-hydroxyquinoline (found C=67·89%, H = 4·45%, N = 19·23%; required C = 68·23%, H = 4·30%, N = 19·90%). For 5, 5′-(*o*-dianisidinedisazo)-8-hydroxyquinoline the formula is $C_{32}H_{24}N_6O_4$ (found C= 68·52%, H = 4·42%, N = 14·97%; required C = 69·05%, H = 4·35%, N = 15·10%).

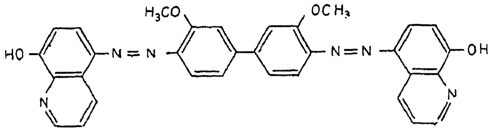
The i.r. spectra (in Nujolmull) shows bands at 3100, 1570, and 1375 cm^{-1} corresponding to O–H stretching, N=N stretching and C–N vibrations[4] respectively in 5, 5′-(*p*-phenylenediaminedisazo-8-hydroxyquinoline

In 5, 5′-(*o*-dianisidinedisazo)-8-hydroxyquinoline, bands are observed at 3105, 1572 and 1370 cm^{-1}, corresponding to the above three assignments, as well as at 1220, 1020 , 430 cm^{-1} due to OCH_3.

On the basis of structure proposed by Boyd[1] for the disazo dye derived from 8-hydroxyquinoline and benzidine, the structures of the dyes reported here are



I. 5, 5′-(*p*-phenylenediaminedisazo)-8-hydroxyquinoline.



II. 5, 5′-(*o*-dianisidinedisazo)-8-hydroxyquinoline.

The coupling occurs on position five, *i.e.*, *p*- to the OH group of 8-hydroxyquinoline and not at position seven *i.e.*, *o*- to the OH group[5]. This is evident because though the *o*- and *p*- positions are sites of high electron density, *p*- is favoured[6,7] because of the following factors : (i) Steric effect due to large entering group[8] (tetrazonium electrophile), prevents coupling at position seven. (ii) Polarisability effect (+ E) created by the electrophile selectively activates *p*-position[9]. (iii) Inductive effect in the phenoxide ion (— I) through its direct effect create an electrostatic potential difference between *o*- and *p*-positions making *p*-more rich in electron density[10]. The above two effects result in the establishment of considerable electron density difference, and the tetrazonium cation which is very sensitive to small electron density differences[11] due to its lower stability[12], concentrates at the *p*-position. (iv) Furthermore mesomeric effect in the phenoxide ion can give both *o*- and *p*- quinonoid transition state for *o*- and *p*-substitution respectively but since *p*-quinonoid form is much more stable[13] it tends to couple at terminal (*p*-) carbon atom in the conjugated series of double bonds.

Work on the metal complexes of these dyes is in progress and will be reported in subsequent communications.

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1. Boyd, T., Degering, E. F. and Shreve, R. N., *Indian Eng. Chem. Anal. Ed.*, 1938, **10**, 606.
2. Geigy, B. P., 529, 063 *vide* K. Venkatraman (Ed.), *The Chemistry of Synthetic Dyes*, Academic Press, New York 1952, Vol. I, 427,
3. Heller, Cf., *J. prakt. Chem.*, 1908, 77 (2), 190.
4. John, R. Dyer, *Applications of Absorption Spectroscopy of Organic Compounds*, Prentice Hall, New Delhi, 1974, pp. 30, 36, 37.
5. Gutzeit, G. and Monnier, R., *Helv. Chim. Acta*, 1933, **16**, 233, 478.
6. Venkatraman, K., *Chemistry of Synthetic Dyes*, Academic Press, New York, 1952, Vol. 1, p. 415.
7. Reutov, O., *Theoretical Principles of Organic Chemistry*, Mir Publishers, Moscow, 1967, p. 508.
8. Packer, J. and Vaughan, J., *A Modern Approach to Organic Chemistry*, Clarendon Press, Oxford, 1958, p. 580.
9. Ingold, C. K., *Structure and Mechanism in Organic Chemistry*, Cornell University Press, New York, 1953, p. 264.
10. —, *Ibid.*, 1953, p. 267.
11. Peter Sykes, *A Guide Book to Mechanism in Organic Chemistry*, Orient Longman, Calcutta, 1965, p. 133.
12. Snow, *Ind. Eng. Chem.*, 1932, **24**, 1420.
13. de la Mare, P. B. D., *J. Chem. Soc.*, 1949, p. 2871.

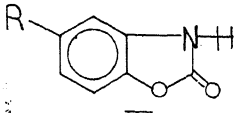
NEW METHODS FOR THE SYNTHESIS OF BENZOXAZOLIN-2-ONES

Two new synthetic methods have been developed to give good yields of benzoxazolin-2-ones (I).

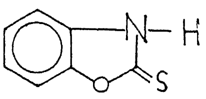
Benzoxazolin-2-ones (I) are biologically versatile compounds[1–5]. The reaction of phosgene[6] with *o*-aminophenols or their fusion with urea[7] are the general methods for obtaining them ; while these methods give good yields of unsubstituted benzoxazolin-2-one, the yields are generally lower in the case of substituted derivatives. In addition, the use of phosgene is hazardous. We wish to report two new methods for the synthesis of I.

In the first procedure (A) *o*-aminophenol (0·1 mole) and urea (0·11 mole) were refluxed with excess of dry pyridine for about 14 hrs. Working up the reaction mixture furnished I (R = H) as a crystalline solid, m.p. 136–138° [6,7] in 80% yield. The method was successfully applied for the preparation of substituted I (R = CO_2H, CO_2Me) with excellent results.

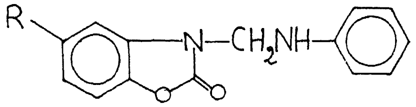
The second method (B) involved the desulfurisation of benzoxazolin-2-thione[8] (III) in DMSO. The latter (0·01 mole) in DMSO containing 5 drops of conc. H_2SO_4 was kept at room temperature for 2 weeks.



I



III



II

Benzoxazolin-2-one (I, R = CO_2Me) was prepared similarly by methods A and B. In another method 5-carboxy-benzoxazolin-2-one (1·79 gm, 0·01 mole), dry methanol (15 ml) and thionyl chloride (1·5 ml) were refluxed for 12 hrs. The solvent was removed and the residue neutralised with 5% sodium bicarbonate. The product was recrystallised (water), m.p. 197^0 (Lit.[9], m.p. 196·5°), yield 1·46 gm (76%). The product obtained was identical with that obtained by methods A and B (m.p., m.m.p., I.R. and T.L.C.) ; I.R. (KBr) : ν_{max} = 3210(NH), 1780 (C=O, ring), 1690 cm^{-1} (C=O, ester). Calcd. for $C_9H_7NO_4$, N = 7·25, Found N = 7·51 %.

5-Carbomethoxy-3-phenylaminomethylbenzoxazolin-2-one (II, R = CO_2Me)

Aniline (0·93 gm) and formalin (1 ml) were added to a boiling ethanolic suspension of I (1·93 gm, R = CO_2Me) with shaking. The reaction mixture was stirred for 10 min. and the product was recrystallised from ethanol, m.p. 188–189° yield 2·0 gm (70%); I.R. (KBr): ν_{max}=3400 (NH), 1760 (C=O, ring) 1700 cm^{-1} (C=O, ester) ; N.M.R. ($CDCl_3$) : σ= 3·90 (Me). 4·74 (CH_2), 5·30 (NH), 6·70–7·90(Ar–H). Calcd, for $C_{16}H_{14}N_2O_4$, N = 9·39. Found N = 9·39%.

II (R = CO_2Me) prepared from I (R = CO_2Me) obtained by all the three methods was found identical (m.p., m.m.p., I.R. and T.L.C.).

5-Carboxybenzoxazolin-2-one (I, R = CO_2H)

Method A : 3-Amino-4-hydroxybenzoic acid (15·3 gm, 0·1 mole) and urea (6·60 gm, 0·11 mole) were refluxed in 30 ml of dry pyridine for 14 hrs. The product thus obtained was recrystallised from ethanol, m.p. > 297^0 ; (Lit.[10], m.p. 336–338^0), yield 14·6 gm (82%).

Method B: 3-Amino-4-hydroxybenzoic acid (15·3 gm) and urea (8·0 gm) were fused at 150° and kept at this temperature for 4 hrs. Working up the reaction mixture gave the expected product, m.p. > 297 ° , yield 10·7 gm (60%).

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1. Lespagnol, A., Mercier, J. and Lespagnol, C., *Arch. Intern. Pharmacodyn.*, 1953, **94**, 212.
2. Debelams, J. and Lespagnol, C., *Anal. Pharm. France*, 1956, **14**, 778.
3. Smith, R. T., Korn, K. M., Peak, W. P. and Hermann, I. F., *J. Am. Med. Assoc.*, 1956, **160**, 745.
4. Kamijo, K. and Kolle, G. B., *Proc. Soc. Exper. Biol. Med.*, 1955, **88**, 565.
5. Varma, R. S. and Nobles, W. L., *J. Pharm. Sci.*, 1968, **57**, 39.
6. Close, W. J., Tiffany, B. D. and Spielman, M. A., *J. Am. Chem. Soc.*, 1945, **71**, 1265.
7. Bywater, W. G., Coleman, W. R., Kamm, O. and Merritt, H. H., *Ibid.*, 1945, **67**, 905.
8. *Organic Syntheses.* N. Rabjohn, Ed., Wiley, New York, N.Y., 1963, Coll. Vol .4, p. 569.
9. Einhorn, A. and Ruppert, E., *Ann.*, 1902, **325**, 324.
10. Clark, R. L. and Pessolano, A. A., *J. Am. Chem. Soc.*, 1958, **80**, 1662.

THERAPEUTIC EVALUATION OF ANTIMYCOTIC DRUGS IN REPEAT BREEDING BOVINES DUE TO MYCOTIC INFECTIONS

THE role of mesophilic and thermophilic fungi in causing repeat breeding, metritis, abortion in females and seminal vesiculitis and orchitis in males has recently been recognised by Zeverva and Repko[1], Saxena and Pathak[2] and Ainsworth and Austwick[3].

In the present investigation an attempt has been made to evaluate a broad spectrum antifungal drug that could be effective against fungi found in repeat breeders.

Fungi were isolated to the extent of 83% from mucopurulent discharges of 92 repeat breeders on Sabouraud's dextrose agar (Saxena[4] and Lachenicht and Potel[5]). They included *Aspergillus* 21% (*Aspergillus fumigatus*, *A. niger*, *A. terrus*, *A. cheveleri*, *A. flavus*), *Candida albicans* and other *Candida* species 6%, *Cladosporium* 10%, *Penicillium* 6%, *Alternaria* 3%, *Mucor* 9%, *Rhizopus* 6%, *Geotrichum* 2%, *Cryptococcus* 1%, *Curvularia* 8%. Other typed fungi were 11% (*Phialaphora*, *Sporotrichum*, *Scopulriopsis*, *Botrytis*, *Allescheria* and *Fusidium*) closely matching the isolates of Indian soil. No fungi could be isolated from 17% cases of repeat breeders.

In vitro drug sensitivity was tried against *A. cheveleri*, *A. terrus*, *A. fumigatus*, *Fusidium*, *Candida* and *Curvularia* species of fungi with 18 drugs (Table I) and the zone of growth inhibition recorded (as slight and marked) after the technique of Grigorin and Grigorin[6]. Only three drugs, useful *in vitro* drug sensitivity test, were tamponed as 1% solution in the vagina and cervix of repeat breeders, at four days intervals in three groups and the result of therapeutic efficacy compared by the conception of animals (Table II). These three groups had all the fungal isolates as noted above from their cervicouterine discharges.

Out of the 18 compounds tested in *in vitro* (Table I) 1% solution of copper sulphate and mercurochrome had the same fungicidal activity as possessed by the expansive drugs Otamadyl (Dibromopropamadine isoethionate 15% and diamidinodiphenylamine dihydrochloride 5%) and Talsutin ovulets (Amphotracin B 50 mg and Oxytetracycline 100 mg). Mycostatin (Nystatin) was slightly inhibitory to the growth of *Fusidium* and *Candida albicans* species. Crystal violet, potassium permanganate lotion and Ampicillin had growth inhibitory effect against *Candida* only while Lugol's iodine the most widely used vaginal antiseptic in veterinary practice had very slight effect against *Aspergillus* species.

Apart from the antifungal activity shown against limited species of fungi in the *in vitro* study, copper sulphate and mercurochrome appeared clinico-therapeutically efficacious to the extent of 78% and 56% respectively in repeat breeders against other isolates also (*Penicillium*, *Alternaria*, *Mucor*, *Rhizopus*, *Allescheria boydii*, *Scopulriopsis* and *Phialaphora*) present in the uterus and not tested in the *in vitro* test. One repeat breeder cow with *Cryptococcus neoformans* isolation responded well to 1% crystal violet intravaginal tamponing (Table II).

The intravaginal application of these cheap antiseptics may find wide use in overcoming repeat breeding problem in animals. Earlier workers (Ciszowski[7], and Saxena[8]), have also commented over the superiority of copper compounds in fungicidal activity. Talsutin infusion in 15 ml distilled water after the technique of Sachii *et al.*[9] has also given encouraging results in reducing bacterial as well as fungal infection of the repeat breeders.

TABLE I

In vitro *drug sensitivity against fungi*

Drugs	Fungi isolated from repeat breeders					
	A. cheveleri	*A. terrus*	*A. fumigatus*	*Fusidium*	*Candida albicans*	*Curvularia*
Otamadyl (M and B)	M	M	M	..	M	..
Talsutin (Squibb)	M	M	M	S	S	..
Copper sulphate 1%	M	M	M	S	M	..
Mercurochrome 1%	M	M	M	S	M	..
Sodium chloride 1%	S	M	S	S	S	S
Potassium permanganate lotion 1 : 1000	..	..	..	..	S	..
Lugol's iodine 1%	S	S	S	..	..	..
Mycostatin 20 mg/ml	..	..	..	S	S	..
Crystal violet 1%	..	..	..	..	S	..
Ampicillin 20 mg/ml	..	..	..	..	S	..
Lactic acid 1% potassium metabisulphite 1% sodium metabisulphite 1% sodium thiosulphate 1–4% benzyl benzoate 1% Nebasulph. (Pfizer) Grisofulvin 20 mg/ml Bifuran (SKF)	..	..	..	..	..	..

M = Marked, S = Slight ; .. = Not sensitive.

Copper sulphate and mercurochrome both as 1% aqueous solutions and Otamadyl (M and B) intrauterine tamponing were found to possess antimycotic therapeutic efficacy to the extent of 78%, 56% and 50% respectively in preventing mycotic repeat breeding. Their antimycotic spectrum appeared against *Aspergillus cheveleri*, *A. fumigatus*, *A. tetrrus*, *Candida albicans*, *Fusidium* in *in vitro* drug sensitivity trials and, in addition, against infections of *Penicillium*, *Alternaria*, *Mucor*, *Rhizopus*, *Allescheria boydii*, *Phialaphora* and *Scopulriopsis* species of fungi in repeat

TABLE II

Clinical trials against fungal repeat breeding cases

Number of repeat breeding cases		Fungal isolates	Drug tried	Results
Cows	Buffaloes			
2 (Brand Nos. 702 and 351)	7 (Brand Nos. 321, 43, 394 37, 393, 145 81)	*C. albicans*, *Penicillium*, *Alternaria*, *Mucor*, *Aspergillus*, *A. terrus*, *Rhizopus*, *Phialaphora*, *Curvularia*	Copper sulphate 1% tamponing	Pregnancy restored and calved
1 (155)	2 (376, 146)	*Aspergillus*, *Alternaria*, *Rhizopus*, *Scopulriopsis*	—do—	Chronic cases of cervicitis; did not respond; non-pregnant
2 (125, 141)	2 (146, 74)	3 *Aspergillus* species *Geotrichum*, *Candida*, *Penicillium*	Mercurochrome 1%	Cases of cervicitis; non-pregnant
...	4 (410, 93, 94, 425)	*Aspergillus*, *Allescheria boydii*, *Scopulriopsis*, *A. terrus*	—do—	Pregnant and calved
1 (884)	..	*Aspergillus* and *Penicillium*	Otamadyl	Cervicitis, non-pregnant
1 (136)	..	*Aspergillus*, *Mucor*	—do—	Pregnant and ealved
..	2 (403, 385)	*A. terrus*, *Penicillium*	Control	Remained repeat breeder
..	2 (144, 83)	*Mucor* and *Curvularia*	—do—	Pregnant and calved
1 (Private)	..	*Cryptococcus neoformans*	Crystal violet	Pregnant and calved

breeding bovines. A single case of Cryptococcal repeat breeding cow responded well to 1% crystal violet intrauterine tamponing.

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---

1. Zaverva, G. V. and Repko, A. N., 2208 *Rev. Med., Vet. Mycol.*, 1968, 7, 35.
2. Saxena, S. C. and Pathak, R. C., *Agra Univ. Res. (Sci.) J.*, May 1972, **21** (2), 21.
3. Ainsworth, G. C. and Austiwick, P. K. C., *Fungpl Diseases of Man and Animal*, Commonwealth Agric. Bureaux, Faruham, Bucks, England, 1959, 1973.
4. Saxena, S. C., *Ph.D. Thesis*, Magadh University, Bodh Gaya, India, 1972.
5. Lachenicht, P. and Potel, J., *Medsche Klin*, 1969, **64** (5), 206.
6. Grigorin, D. and Grigorin, A., *Mycopath. Appl.*, 1970, **41**, 373.
7. Ciszewski, K., *Medyeyna Wet.*, 1968, **24**, 150.
8. Saxena, S. C., *M.V.Sc. Thesis*, Agra University, Agra, India, 1966.
9. Sacchi, E. M., Smith, E. B. and Tower, J. H., *New York Medical Encyclopedia Inc. Vet. Bull.*, 1958, **29**, 890.

BLOOD COPPER CONTENT AND MINIMUM MOLECULAR WEIGHT OF HAEMOCYANIN OF *CRYPTOZONA LIGULATA* (FERRUSAC)

THERE is extensive literature on the chemistry and biology of the haemocyanins[1-4]. The arthropod and molluscan haemocyanins have been shown to be distinct in their copper content and molecular weights[4]. In the present note an attempt has been made to determine the copper and haemocyanin content and the minimal molecular weight of the haemocyanin in the blood of active, aestivated and naturally revived snail, *Cryptozona ligulata*.

The snails were collected and maintained in the laboratory as described earlier[5]. Haemocyanin was prepared according to the procedure given by Konings *et al.*[6]. But the haemocyanin solutions were dialysed for 24 hours against 25 mm EDTA in 0·1 M Tris-HCl buffer, pH 8·0, the normal blood pH of the snail.

The copper and the protein contents were estimated in the blood as well as in the purified haemocyanin solutions by the methods of Barnes and Rothschild[7] and Lowry *et al.*[8] respectively. Haemocyanin content in the blood was calculated by dividing the blood copper content by the fraction of copper in the purified haemocyanin[1]. The minimal molecular weight of the haemocyanin containing one atom of copper is given by dividing the atomic weight of copper (63·54) by the fraction of copper present in the purified haemocyanin[9].

TABLE I

Copper and protein content in the blood and purified haemocyanin of C. ligulata. *Values are mean ± S.D. of 10 individual observations*

	Active	Aestivated	Naturally revived
Blood :			
Copper (μgm/ml)	31·30 ± 2·76	42·60 ± 3·67*	42·90 ± 4·55*
Total protein (mg/ml)	21·52 ± 3·21	18·59 ± 1·35*	20·26 ± 1·73^{NS}
% Copper in Total protein	0·15	0·23	0·21
Haemocyanin (mg/ml)	12·52	17·00	17·16
Water content (% gm. wt.)	98·23 ± 1·25	90·15 ± 0·95*	98·10 ± 0·59^{NS}
Purified Haemocyanin :			
Copper (μgm/ml)	27·50 ± 1·32	34·80 ± 1·84*	39·00 ± 2·10*
Protein (mg/ml)	11·00 ± 1·75	13·90 ± 0·92*	15·60 ± 1·15*
% Copper in Haemocyanin	0·25	0·25	0·25

* $P < 0·001$. N.S. = Not significant.

From Table I it is clear that in the blood of *C. ligulata*, he total protein content is low during aestivation; here is no significant difference upon revival, suggesting he utilisation of blood proteins during aestivation.

The blood copper content inercased during aestiation and also upon revival (Table I) and the haemoyanin content followed the same pattern. The increase n copper content of aestivated snails could be due ither to the dehydration of blood by 8·1% (Table I) r to the release of copper from the hepatopancreas hich is known to store copper[10,11]. In the naturally evived snails, even though the water content in the lood is restored almost to the normal level, the copper ontent is higher as in the blood of aestivated snails, uggesting that more haemocyanin is needed for evival.

During aestivation the snails live in low ambient xygen tension[12]. The extraction efficiency of oxygen increases with decreasing oxygen tension in the Ampullarid snail, *Marisa cornuarietis*[13]. In *C. ligulata*, the increased concentration of haemocyanin (36·1%) during aestivation suggests that the blood has a greater binding capacity for oxygen in hypoxic condition[2]. During natural revival the haemocyanin concentration is maintained which suggests greater oxygen transport to meet the elevated metabolic rate[14] in order to attain the normal physiological state.

The per cent ratio of copper/total portein in the blood of *C. ligulata* is different in the three conditions (Table I). In the purified haemocyanin of *C. lingulata*, the copper content is 0·25% in the active, aestivated and naturally revived snails (Table I) and this is in agreement with the other gastropod haemocyanins[4] (Table II).

TABLE II

Copper content and the minimal molecular weight of the haemocyanin of C. ligulata *compared with other gastropods*[4]

Species	% Cu	M/Cu	Ref.
Busycon canaliculatum	0·245	25,900	15
Helix pomatia	0·250	25,400	16
Murex trunculus	0·257	24,700	17
**Cryptozona ligulata*	0·250	25,420	..

* Present investigation,

The minimal molecular weight of the haemocyanin of *C. ligulata* is 25,420 (Table II), estimated upon the basis of its copper content $(0\cdot25 \times 10^{-2})$. One molecule of oxygen combines with a functional unit of haemocyanin containing two atoms of copper[3]. On this basis, the minimal molecular weight of the functional unit of haemocyanin of *C. ligulata* would be 50,840. From Table II it is clear that the functional units of gastropod haemocyanins are almost similar.

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---

1. Prosser, C. L. and Brown, F. A., *Comparative Animal Physiology*, 2nd Ed., W. B. Saunders Co., Philadelphia, 1961, Chap. 8, p. 198.
2. Manwell, C., *A. Rev. Physiol.*, 1960, **22**, 191.
3. Redmond, J. R., In: *Chemical Zoology*, Ed. by Florkin, M and Scheer, B. T., Vol VI. Arthropoda, Part B, A.P., New York, 1971, Chap. 3, p. 119.
4. Lontie, R. and Witters, R., In: *Inorganic Biochemistry*. Ed. by Echiron, G. L., Elsevier, Amsterdam, 1973, Chap. 12, p. 343.
5. Krupanidhi, S. and Padmanabha Naidu, B., *Com. Physiol. Ecol.*, 1977, **2** (2), 54.
6. Konings, W. N., Van Driel, R., Van Bruggen, E. F. J. and Grubber, M., *Biochim. Biophys. Acta*, 1969, **194**, 55.
7. Barnes, H. and Lord Rothschild, *J. Exp. Biol.*, 1950, **27** (2), 123.
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
9. Montgomery, H., *Biol. Bull.*, 1930, **58**, 18.
10. Wieser, W., *Am. Zoologist*, 1968, **8**, 495.
11. Djangmah, J. S., *Comp. Biochem. Physiol.*, 1970, **32**, 709.
12. Von Brand, T., *Biodyanamica monographs*, Normandi, 1946, p. 328.
13. Akerland, G., *Comp. Biochem. Physiol.*, 1974, **47A**, 1065.
14. Herreid. C. F., *Ibid.*, 1977, **56A**, 211.
15. Hernler, F. and Philippi, E., *Z. Physiol. Chem.*, 1933, **216**, 110.
16. Roche, J., *Essai sur la biochimie generale et comparee des pigments respiratoires*, Masson, Paris, 1936.
17. Ghiretti-Magaldi, A., Nuzzolo, C. and Ghiretti, F., *Biochemistry*, 1966, **5**, 1943.

ISOLATION & BIOLOGICAL ACTIVITY OF A STRAIN OF *STREPTOMYCES GANMYCICUS* AND STABILITY OF THE ANTIBIOTIC SUBSTANCE PRODUCED BY IT

This paper deals with a new strain of *Streptomyces ganmycicus* antagonistic to *Colletotrichum graminicola* (Ces.) Wilson inciting anthracnose in sorghum.

Morphology and Cultural Characteristics

An actinomycete (Isolate No. S–15) was isolated from the soil of cultivated fields of Sikandra at Agra adopting dilution plate technique[6] on Thornton's agar[1].

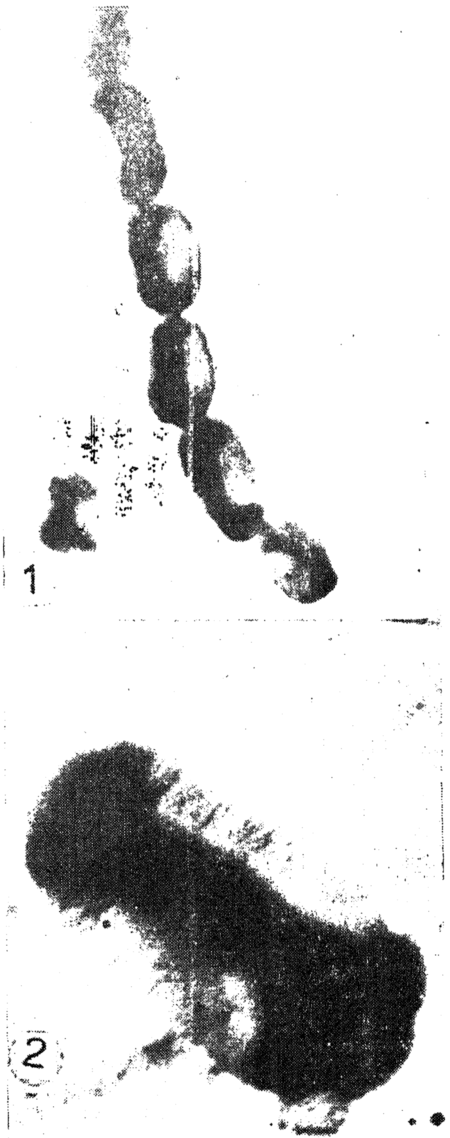
The actinomycete (S–15) is a chromogenic type, produces soluble pigment on natural media, is melanin positive and shows acidic reaction in milk. It slowly liquifies gelatin, hydrolyses starch and reduces nitrate completely. It utilizes best the glucose, maltose and rhamnose as the carbon sources, whereas arabinose and xylose were poorly utilized.

Vegetative mycelium is white, non-septate and monopodially branched forming tough textured growth on solid media. Aerial mycelium is abundant, rosy white to grey in colour, sporophores as extended long and open spirals. Spores in chains (Fig. 1), are oval to short cylindrical, 1·0–1·6 × 1·6–2·2 μ, wall spiny when examined under electron microscope (Fig. 2). The isolate S–15 was placed in section "Spira" and "Grey" series[3].

The strain S–15 resembles *S. ganmycicus* in surface configuration of spores, which is considered a constant and reliable taxonomic criterion for the classification of *Streptomyces* species[4, 5, 7]. However there are some differences between the two in cultural characteristics. In sucrose-nitrate medium S–15 does not produce pale yellow soluble pigment like *S. ganmycicus*. Similarly on starch agar, vegetative mycelium in *S. ganmycicus* is yellowish brown[2] whereas it is whitish brown in S–15. Therefore Isolate S–15 has been considered as a new strain of *Streptomyces ganmycicus* Hosoya and Soeda. The validity of identification has been confirmed by reference to the Central bureau voor Schimmelcultures, Baarn, The Netherla .

Biological Activity

The new strain of *S. ganmycicus* was found to possess marked antagonistic activity against *Colletotrichum graminicola*. The activity of the strain was further tested by streak method against other micro-organisms. The culture was inoculated by spore suspension as a broad streak about the edge of the petri plate, on potato-dextrose agar medium. After three days of incubation at room temperature the different micro-organisms were streaked at right angle to the actinomycete streak. The inhibition zone if formed was measured. Thus it was found that the new strain of *S. ganmycicus* was also active against *Altenaria alternata*, *A. brassicae*: *Aspergillus erythrocephalus*, *A. flavus, A. flavipes, A. fumigatus, A. nidulans, A. niger, A. sydowii, A. tamerii, A. terrus*; *Chaetomium spirale*; *Cladosporium cladosporoides*; *Cochliobolus specifier*; *Cunninghamella echinulata*; *Curvularia prasadi, C. tuberculata, C. verruculosa*; *Drechslera halodes, D. hawaiiensis, Drechslera* state of *Cochliobolus sativus, Drechslera* state of *Trichometasphaerea holmii*; *Fusarium concolor, F. equiseti, F. fusaroides, F. heterosporium, F. moniliforme, F. oxysporum, F. semitectum*; *Mucor hiemalis*; *Nodulosporium griseobrunneum*; *Penicillium citrinum, P. luteum, P. spiculisoporum, P steckii*; *Stachybotrys atra*; *Trichothecium roseum*; *Trichoderma lignorum Bacillus subtilis* and *Staphylococcus* sp.



FIGS. 1–2. Fig. 1. Electron microphotograph of a new strain of *S. ganmycicus* showing a spore chain. Fig. 2. Electron microphotograph of *S. ganmycicus* showing a spore with spiny wall.

Stability of the Antibiotic Substance

The antibiotic produced by the new strain of *S. ganmycicus* is thermolabile. It loses its activity at temperatures of 50° C and above or when diluted with water. Upon storage the activity is also lost, the loss being more rapid at high temperature (30–35° C). pH of the culture broth also influences its activity. It is lost both at higher (8·5) and lower (4·5) ranges but more rapidly in the acidic side. The antibiotic can be stored without any appreciable loss in its activity for 36 days at pH 7·6 and temperature range 0–7° C.

The antibiotic is readily sholuble in *n*-butanol and differs from carzinomycin produced by *S. ganmycicus*. The antibiotic produced by S-15 is a non-polyenic, polymeric product of amino sugars containing $-NH_2$ and $-OH$ groups.

The authors wish to express their thanks to the Director of JALMA, Agra, for electron microphotography, to the Director, Centraalbureau voor Schimmelcultures, Baarn, for identification, and to University Grants Commission for financial help.

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Varanasi, India, *August* 4, 1977.

1. Allen, O. P., *Experiments in Soil Bacteriology*. Burgess Publishing Co., Minnesota, 1951.
2. Harada, Y. and Tanaka, S., "Studies on carzinocidin, antitumor substance produced by *Streptomyces* sp. III. On the taxonomic studies of the strain No. 48-B-3 identified as *S. kitazawaensis* Nov. sp. *Antibiotic (Japan)*, 1956, **9A**, 112.
3. Pridham, T. C., Hesseltine, E. W. and Benedict, "A guide for the classification of Streptomycetes according to selected groups." *Appl. Microbiol.*, 1958, **6**, 52.
4. Tresner, H. D., Backus, E. J. and Hayer, J. A., "Morphological spore types in *Streptomyces hygroscopicus* like complex," *Ibid.*, 1967, **15**, 637.
5. —, Davies, M. E. and Backus, E. J., "Electron microscopy of Streptomyces spore morphology and its role in species differentiation," *J. Bacteriology.*, 1961, **81**, 70.
6. Waksman, S. A., *Principles of Soil Microbiology*, Willams and Wilkins Co., Baltimore, 1927.
7. Williams, S. T., Further investigations of actinomycetes by scanning electron microscopy," *J. gen. Microbiol.*, 1970, **62**, 67.

TWO NEW SPECIES OF HYPHOMYCETES

Two new species of Hyphomycetes, viz., *Heteroconium lignicolum* and *Taeniolella lignicola* are being described from Mount Abu, Rajasthan.

(1) *Heteroconium lignicolum* Panwar & Chauhan sp. nov.

Coloniae effusae, brunneae ad fuscas, mycelium plerumque superficiale, hyphae 2·7–5·4 μ crassae, brunneae; Conidiophora ex hyphislateraliter exorienta, nonramosa recta vel flexuosa, ferruginae ad brunnea in colore, laevia usque ad 25 μ longa et 4–5·5 μ crassa. Cellulae conidiogenae, monoblasticae, integratae, terminales, cylindricae; Conidia apicibus, conidiophororum portato catenata, simpliciae, cylindrica cum extremis truncatis seu rotundatis, usquead brunnea 1–4 septata, laevia 13·5–32·5 × 5·4–8·5 μ (Fig. 1).

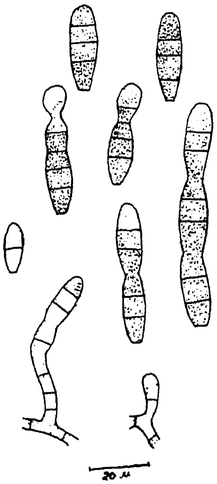

FIG. 1

Typus lectus in lingo emortuo in loco Mount Abu die October 1976 a, K. S. Panwar et J. S. Chauhan et positus C.M.I., Kew Herb IMI 199273 typus et Botany Department, University of Jodhpur, Coll. J.U.M.L. 613.

Colonies effuse, brown to blackish brown. Mycelium mostly superficial, hyphae 2·7–5·4 *u* thick, pale-brown. Conidiophores arise laterally from the hyphae, unbranched, straight or flexous, mid to dark brown, smooth, upto 25 μ long and 4–5·5 μ thick. Conidiogenous cells monoblastic, integrated, terminal cylindrical. Conidia borne at the tips of conidiophores, catenate, simple cylindrical with truncate or rounded ends, mid to dark brown, 1 to 4 septate, smooth, 13·5–32·5 × 5·4–8·5 *u*.

Collected on dead twigs from Mount Abu by K. S. Panwar and J. S. Chauhan on October 1976 and deposited in C.M.I., Kew Herb I.M.I. 199273 and Botany Department, University of Jodhpur, Coll. J.U.M.L. 613.

The present fungus is quite distinct from the four known species of this genus in the shape, size and septation of the conidia. However, it comes close to *Heteroconium solaninum* (Sacc. & Syd.) M. B. Ellis in the septation and size of conidia but differs in the shape of conidia and the size of conidiophore.

(2) *Taeniolella lignicola* Panwar and Chauhan sp. nov.

Coloniae effuse fuligineae. Mycelium paucum parte superficiale et parteimmersum in substrato, 2·5–4·5 μ crassum, brunneum, sine conidiophoris. Conidia cellulis conidiogenis exorientia, quae directer producentur a mycelio obsoleto lateralifer, sicut ac terminaliter. Conidia, catenulata cum simplicubus vel ramosis catenis. Conidia recta seu flava, truncata apud uteosque terminoscum septa transversa crassa 8–22 27–108 × 12–15 μ (Fig. 2).

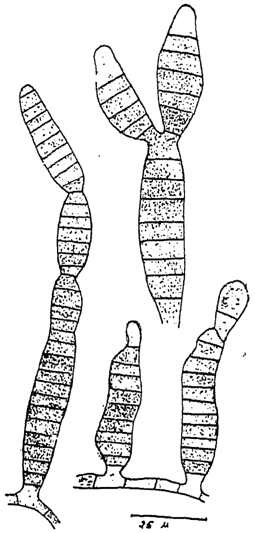

FIG. 2

Typus lectus in lingo emortuo in loco Mount Abu die September 1974 a K. S. Panwar et J. S. Chauhan et positus. C.M.I., Kew Herb IMI 189377 (B) typus et Botany Department, University of Jodhpur, Coll. J.U.M.L. 427 (B).

Colonies effuse, dark-brown. Mycelium scanty, partly superficial and partly immersed in the substratum, 2·5–4·5 *u* thick, dark-brown. Conidiophore absent. Conidia arise from conidiogenous cells which are directly produced by the obsolete mycelium laterally as well as terminally. Conidia catenulate, the chain may be simple or branched. Conidia straight or flexuous, truncate at both ends having 8 to 22 thick transverse septa, 27–108 × 12–15 μ.

Collected on dead wood from Mount Abu by K. S. Panwar and J. S. Chauhan on September 1974 and deposited in C.M.I., Kew Herb I.M.I. 189377 (B) and Botany Department, University of Jodhpur, Coll. J.U.M.L. 427 (B).

The present fungus comes close to *Taeniolella rudis* (Sacc.) Hughes but differs in the size and septation of the conidia, moreover the conidia are produced in branched chains.

The authors are grateful to Dr. M. B. Ellis and Dr. B. C. Sutton for the help in the identification of fungi. Thanks are due to Prof. H. C. Arya for providing laboratory facilities and to Rev. Father William Barracos for the Latin translation. The financial assistance received from C.S.I.R., New Delhi, is gratefully acknowledged.

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---

1. Ellis, M. B., *Dematiaceous Hyphomycetes*, Commonwealth Mycological Institute Publication, England, 1971.
2. —, *More Dematiaceous Hyphomycetes*, Commonwealth Mycological Institute Publication, England, 1976.

A NEW *AECIDIUM* FROM BURMA

SINCE no rust has so far been reported on *Artabotrys* (Fam. Annonaceae) (Laundon—personal communication 1975) and rust fungi are obligatory host-specific parasites, the following new specific taxon is proposed to accommodate the rust infecting *Artabotrys* sp.

Aecidium artabotrydis Thaung sp. nov. (Fig. 1)

Spermogoniis epiphyllis, in maculis flavo-aurantiis necroticis infectionis aeciis intermixtis, subepidermalibus, late ad latissime obovatis, usque 150 μm latis et 140 μm altis, ex typis 1 sensu Hiratsukaeae et Cumminsii (1963). *Aeciis* amphigenis, in gregibus dispositis inter vel sine spermogoniis, subepidermalibus, erumpentibus, plerumque cupulatis, usque 185–259 μm latis, 370–630 μm altis; cellulis peridii luteolis, ellipsoideis, 26–31·5 (–35) × 10–15 (–16·5 μm), parietie exteriori usque 3·5 μm crasso, valde sculpto; aeciosporis globoideis vel polygoniis, (13–) 15–18·5 (–20·5) × 14–15 (–17 μm); membrana 1–2·5 μm crassa, sulphureis versus luteolo-aurantiacis, verruculosa.

In foliis vivis *Artabotrydis* sp., Thondon village en route Maymyo, Burma, Maung Mya Thaung, 18 May 1975, IMI 194468 holotypus.

Spermogonia epiphyllous, intermixed with aecia in yellow-orange necrotic infection spots, subepidermal, broadly to very broadly obovate, up to 150 μm wide and 140 μm deep, of Type 1 of Hiratsuka and Cummins (1963). *Aecia* amphigenous, disposed in groups with or without spermogonia, subepidermal, erumpent, mostly cupulate, up to 185–259 μm wide, 370–630 μm

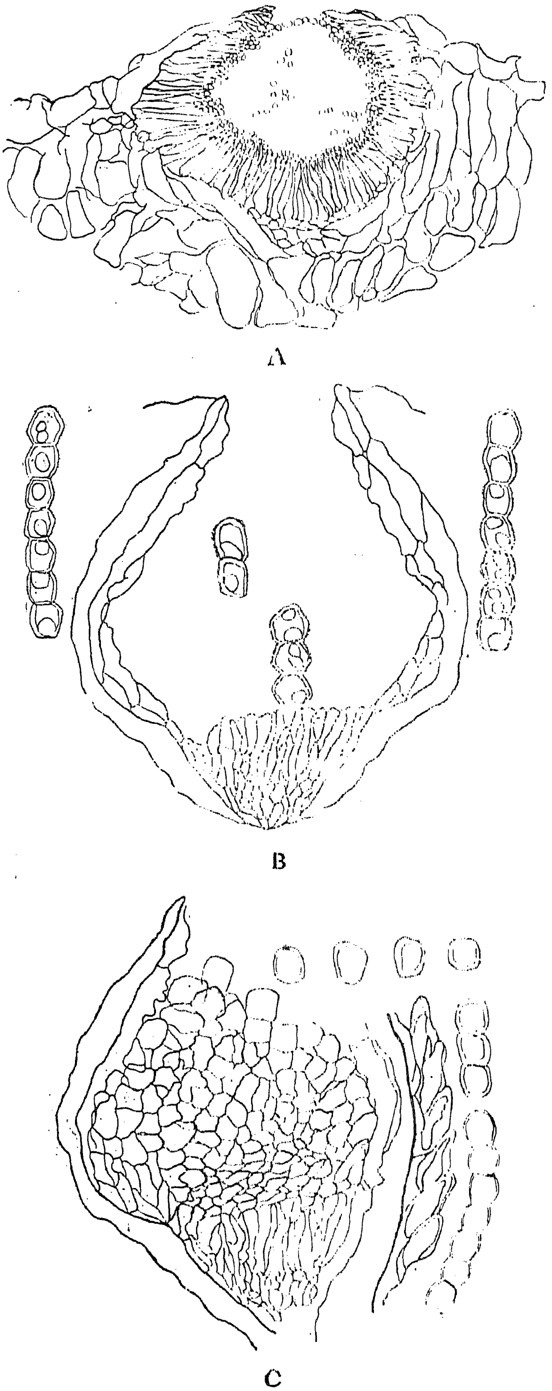

FIG. 1. *Aecidium artabotrydis* (× 400). A. Section through spermogonium on leaf; B. Section through aecium on leaf; C. aeciospores and peridial cells.

deep; peridial cells pale yellow, ellipsoid, 26–31·5 (–35) × 10–15 (–16·5 μm); wall exterior up to 3·5 μm thick, strongly sculptured; aeciospores globoid or polygonal (13–) 15–18·5 (–20·5) × 14–15 (– 17 μm); wall 1–2·5 μm thick, sulphur yellow to pale yellow-orange, verruculose.

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1. Hiratsuka, Y. and Cummins, G. B., "Morphology of the Spermogonia of the Rust Fungi," *Mycologia*, 1963, **55**, 487.

A NEW *TRIMMATOSTROMA* FROM SOIL

DURING the study of ecological distribution of soil fungi, a species of *Trimmatostroma* was isolated from soil samples collected from coffee plantations at Anantagiri (A.P.). On comparison (Ellis.[2,3]) it was found to be a new taxon which is described below. Earlier *T. betulinum* (Corda) Hughes (=*Coniothecium betulinum*) was reported to have been isolated from soils of Ireland (Barron)[1]. The present fungus was also found to differ from *T. cordaicis* (Sharma and Singh[5]) and *T. Hughesii* (Rao and Subedar[4]) described recently from India.

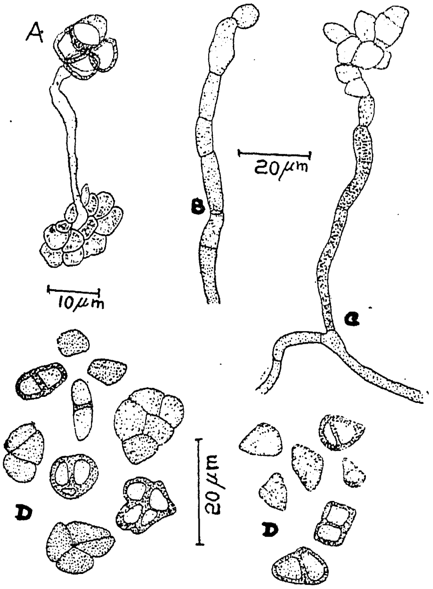

FIG. 1. *Trimmatostroma indica* sp. nov. A. Part of stroma with conidiophore and conidia; B and C. Conidiophore, mycelium with conidiophore and conidia; D. Conidia.

Trimmatostroma indica sp. nov. Manoharachary, Raghuveer Rao and Ramarao.

Coloniae irregulares, gregaria, erumpentia, nigra in medio solanacco agaro sucroso, stromata erumpentia ad celluli hyalina vel subhyalina, producenti conidiophora; conidiophora simplicia vel ramosa rarius, septata, subhyalina, brunnea vel pallide brunnea, usque 150 μm longa, 5 μm lata, usque 5-septata; conidia variabilia, 0–4 septata (7-septata rarius), septa transversalibus vel oblique-longitudinalibus. oriunda, pallide vel fusce brunnea, cylindrica vel ovalia vel triangularis vel quadrangulares, attenuata ad septa, 4·5–15·0 μm longa, 4·5–9·0 μm lata, obtusa vel truncata ad apicem, catenata, formantia catenas, irregulares, ramosa (Fig. 1).

In solo coffee (*Coffea arabica* L.), Anantagiri, Visakhapatnam District, A.P., India, 17 November 1970; pH 8·0; cultura typa posita in C.M.I., Kew, England, IMI 156719, atfue isotypa posita in Mycologia laboratoria, Osmania Universita, Hyderabad, OUF–84.

The authors are greatly indebted to Dr. M. B. Ellis, C.M.I., Kew, England, for help in the identification of the fungus and to Prof. Jafar Nizam, Head of the Botany Department, for encouragement.

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1. Barron, G. L., *The Genera of Hyphomycetes from Soil*, The Williams & Wilkins Co., Baltimore, 1968, p. 364.
2. Ellis, M. B., *Dematiaceous Hyphomycetes*, C.M.I., Kew, England, 1971. p. 41.
3. —, *More Dematiaceous Hyphomycetes*, C.M.I. Kew, England, 1976, p. 27.
4. Rao, V. G. and Subhedar, A. W., *Mycopathologia*, 58, (2), 79.
5. Sharma, N. D. and Singh, S. R., *Curr. Sci.*, 1976, **45** (8), 302.

DETERMINATION OF CAPTAFOL IN ITS FORMULATIONS AND RESIDUES ON CROP PLANTS

CAPTAFOL * (Cis-N-(1, 1, 2. 2-Tetrachloroethylthio), 4-cyclohexene-1, 2-dicarboximide) a broad spectrum fungicide, is generally estimated gas chromatographically or by using a T.L.C. procedure[1]. For routine laboratory analysis of the fungicide and its residues on crops we have attempted to evolve a simple, but sensitive colorimetric method. The method is based on the principle that captafol reacts under alkaline conditions with N, N-dimethyl-*p*-phenylene diamine (DPD), yielding a brownish orange coloured compound that has an absorption maximum at 440 nm. Baumler and Rippstein[2] used this as a spray reagent for identification and estimation of sulphur and chlorine containing organic compounds on T.L.C. plates. It has been possible to use this reagent for quantitative estimation of captafol colorimetrically, as given in this article.

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* Difolatan is the registered trade name of M/s. Chevron Chemical Company for Captafol.

Reagents:

(*i*) DPD reagent—Freshly prepared solution of N, N-dimethyl-*p*-phenylene diammonium dichloride—5 mg/ml of 0·5 N methanolic NaOH.

(*ii*) Standard captafol solution—one μg/ml in benzene.

Procedure:

(*a*) *For formulations*: A weighed quantity (100 mg) of the active ingredient is diluted to one litre with benzene and 5 ml of this solution is pipetted out into a 50 ml test tube, evaporated slowly to dryness on a water bath and the content of captafol is determined by the same procedure as for the preparation of standard curve.

(*b*) *For residues*: The procedures for extraction of captafol residues from crops and subsequent clean up given by Pack[1] is used, keeping in mind that certain crops like apples, celery, peaches, spinach and tomatoes need no clean up. The finely chopped plant parts (100 g) are extracted twice with 400 ml of benzene in a waring blendor, filtering each time under suction. The extracts are evaporated to dryness, dissolved in acetonitrile and transferred to a 125 ml separatory funnel. The acetonitrile extract is washed thrice with 10 ml portions of *n*-hexane and the hexane washings are discarded. The acetonitrile solution is made upto 100 ml. An aliquot of this solution is evaporated to dryness in a vacuum evaporator, the residue dissolved in a small volume of pentane and passed through a 10 cm column of silicic acid. The column is washed 5 times with 5 ml portions of pentane and the fungicide is eluted with six 5 ml portions of ether-pentane (1:1) mixture. The eluate is evaporated in a vacuum evaporator. The residue is dissolved in 10 ml benzene, transferred to a 50 ml test tube and captafol is estimated as under preparation of standard curve.

Preparation of Standard Curve:

To a series of 50 ml test tubes, 0 to 10 ml of standard (1 *ug*/ml) captafol solution is transferred, the solvent is evaporated slowly to dryness on a water bath. After cooling, one ml of the DPD reagent is added and the test tubes are rotated to bring the residue in contact with the reagent. The mixture is allowed to stand for three minutes and 10 ml of methanol is added. After mixing the absorbance of the solution is measured at 440 nm after 10 minutes.

The method is quite sensitive and the limit of detectability is one μg. By suitably increasing the sample size, for residue analysis the sensitivity can be increased upto 0·02 ppm. The recovery of captafol from this method varied from 85 to 110% which is well within the generally accepted limits.

The authors are grateful to the Director (F & P) Rallis India Ltd., for permission to publish this method.

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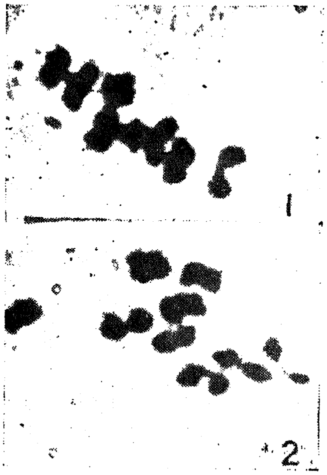
---

1. Pack, D. E., In *Analytical Methods for Pesticides, Food Additives and Plan Growth Regulators* (Ed. G. Zweig), Academic Press, New York, 1967, p. 293.
2. Baumler, J. and Rippstein, S., *Helv. Chim. Acta*, 1962, **44**, 1162.

A NEW CATEGORY OF B-CHROMOSOMES IN *TRIGONELLA FOENUM-GRAECUM* L.

B-CHROMOSOMES smaller than the normal complement have already been reported in *Trigonella foenum-graecum* from this laboratory (Raghuvanshi and Joshi[1]). A new category of B-chromosomes indistinguishable from normal complement is being reported for the first time in this species. The chromosome number of this species is $2n = 16$. In one of the strains carrier plants have two types of PMCs, one having 16 chromosomes (Fig. 1) and other with 18 chromosomes (Fig. 2). These 2 B-chromosomes are



FIGS. 1 and 2. PMCs with 8 and 9 bivalents respectively.

indistinguishable from the normal complement and they regularly pair to from bivalent which generally behave normally during subsequent stages. However, evidence of elimination of chromosomes at certain meiotic stages has been obtained in some cases.

This carrier strain is under study in our laboratory for several years. The progeny of the carrier plant consists of both carriers and non-carriers. But the important point to be noted is that in carrier PMCs of carrier plants these B-chromosomes are generally two in number. Only in rare cases a variation from **1**–3 has been noted. Out of 36 plants studied 23 were carriers. The frequency of PMCs having B-chromosomes in different carrier plants varied from 11·4% to 32·0%.

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Lucknow University, Manjula Upreti.
Lucknow, *August* 26, 1977.

---

1. Raghuvanshi, S. S. and Sheila Joshi, *Curr. Sci.*, 1964, **33**, 654.

NEW GENERA FROM THE EXOTIC BLOCK AT LAMAYURU, LADAKH

Tewari and Pande[1] recorded the present of an exotic block at Lamayuru (34° 17′ 30″ N. : 76° 46′ 31″ E.) in Ladakh district. This exotic block lies within the Dras volcano-sedimentary suite of the Indus belt which is assigned a Cretaceous age on the basis of the presence of *Orbitolina* and *Hippurites*.

Tewari and Pande suggest a "probable Permian" age for this exotic block. The present authors have found additional fauna from this block. The new forms include the genera *Paradoxiella* sp., *Pachyphloia* sp. and *Colaniella* sp. which indicate a definite Upper Permian age for this block. Assemblage of *Pachyphloia* spp. and *Colaniella* spp. have been recently reported from Guryul Ravine in Kashmir[2], while *Paradoxiella* is not known from the Himalayas.

The authors are thankful to Professor T. R. Sharma for facilities and to Professor S. B. Bhatia for help in identification. The first author is thankful to O.N.G. Commission for granting study leave for this work and to Wadia Institute of Himalayan Geology for providing a fellowship.

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Jammu 180 001, *June* 18, 1977.

---

1. Tewari, B. S. and Pande, I. C., *Pub. Cen. Adv. Stud. Goel.*, Chandigarh, 1970, 7(1), 188.
2. Okimura, Y. *et. al.*, *Mem. Fac. Sci. Kyoto Univ. Ser. Goel. & Min.*, 1975, 41(1), 35.

HAEMATOLOGICAL STUDIES OF FRESHWATER TELEOSTS

The morphological and physiological characteristics of fish blood have been studied by earlier workers—Gulliver[1], Margh[2], Bonnet[3], Dhar[4], Banerjee[5], Srivastava[6] etc. in the different species. Recently, Dube[7] studied the RBC counts and haemoglobin content in *Anabas testudineus*. The present communication attempts to report briefly the morphological studies of blood in *Labeo rohita*, *Labeo calbasu*, *Mystus seenghala* and *W. attu*.

Fishes of different weight ranging from 1·5 to 4·0 kg were collected from local ponds during summer 1975. Blood was collected from the heart after exposing it. Total RBC and WBC counts, Differential leucocyte counts (DLC), Haemoglobin content (Hb) and specific gravity of blood were determined by the standard methods described by Dacie and Lewis[8].

In the present investigation the RBC were found to be oval as well as circular. The average total numbers of RBC were found 3·37 and $2{\cdot}97 \times 10^6/\mathrm{mm}^3$ *L. rohita* and *L. calbasu* and 3·57 and $4{\cdot}17 \times 10^6/\mathrm{mm}^3$ in *M. seenghala* and *W. attu* respectively. The variation observed may be due to the ecological factors such as oxygen requirement of the species, oxygen tension of the environment and effificiency of the oxygen (Hall and Gray[9]).

The average total numbers of WBC in *L. rohita*, *L. calbasu*, *M. seenghala* and *W. attu* were found 14,000 18,000, 14,000 and 12,000/mm^3 respectively. The counts are apparently higher than reported by Bagchi and Ibrahim[10].

The mean data for the DLC showed, lymphocytes (82·5, 86·0, 80·0 and 73·0%); neutrophils (13·0, 11·0, 15·0 and 21·0%); basophils (1·0, 1·0, 1·0 and 1·5%); Eosinophils (2·0, 1·0, 2·0 and 2·5%) and monocytes (1·5, 1·0, 2·0 and 2·0%) in *L. rohita*, *L. calbasu*, *M. seenghala* and *W. attu* respectively. The Variation observed in the DLC may be due to the physiological and ecological state of the fishes (Smith *et al.*[11]).

The average Hb content were found 10·8 and 8·2 gm% in *L. rohita* and *L. calbasu* and 9·2 and 11·6 gm% in *M. seenghala* and *W. attu* respectively. These values are well compared with those found by Pradhan[12] and Qayyum and Naseem[13].

The values for the specific gravity of plasma and whole blood were found to be similar, *i.e.*, 1·055 and 1·025 respectively in the fishes examined with the exception of *L. rohita* where the sp. gr. of plasma was observed to be 1·035. These values compare well with those of Bagchi and Ibrahim[10].

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1. Gulliver, G., *J. Zool. Soc. Land.*, 1875, p. 474
2. Marsh, M. C., *Wash. Med. Ann.* 1902, **1**, 397.
3. Bonnet, V., *J. Physiol. et Path. Gen.*, 1929, **27** (4); 735.
4. Dhar, R. P., *Proc. Zool. Soc. Bengal.*, 1948, **1**, 67.
5. Banerjee, V., *Curr. Sci.*, 1957, **26**, 58.
6. Srivastava, A. K., *Anat. Anz.*, 1968 *a*, **123** (3), 233.
7. Dube, S. C., *Folia Haematol* (*Leipez*), 1974, **100** (4), 436.
8. Dacie, J. V. and Lewis, S. M., *Practical Haematology*, 4th ed. J. and A. Churchill Ltd., London, 1969.
9. Hall and Grey, *J. Biol. Chem.*, 1929, **81**, 589.
10. Bagchi, M. M. and Ibrahim, K. H., *J. Inland Fish Soc. India*, 1974, **6**, 93.
11. Smith, C. G., Lewis, W. M. and Kaplan, H. M., *Prog. Fish Cult.*, 1952, **14** (4), 169.
12. Pradhan, V., *Proc. Indian Acad. Sci.*, 1961, **54 B** : 251.
13. Qayyum, A. and Naseem, S. M., *Curr. Sci.*, 1967, **36** (16), 435.

VOMITING RESPONSE IN THE INDIAN FROGS *RANA TIGRINA* (DAUD) AND *RANA CYANOPHLYCTIS* (BOULENGER)

Introduction

RETROGRADE expulsion of stomach contents by vomiting is very well known in birds and in a large number of mammals. Pigeons of both sexes develop 'cytogenic crop glands' in their crops under the influence of gonadotropic pituitary hormones. Crop glands produce the 'pigeon's milk' which is regurgitated into the mouths of the chickens. Lower Chordates such as the lancelets (*Branchiostoma*) have the capacity to produce a '*rejection current*'. When after a couple of minutes of filtering the food from the water current, sand particles begin to obstruct the incoming water current. This rejection current is produced by the sudden contraction of transverse muscles of the atrial floor, causing the atrial opening to close and raising the atrial floor, thereby compressing the pharynx, which in turn ejects the water through the enterostome. There is a long evolutionary gap between the lower chordates and the birds and the mammals so far vomiting is concerned[1,6]. Attempts were made[8,12] to establish vomiting in the newts, frogs and toads. Both workers experimented upon fully pithed frogs after opening of the abdominal cavity. They also used subcutaneous and intragastric injections of certain emetics to bring about vomiting in frogs and toads in which brain and spinal cords were damaged. They, however, failed to induce vomiting in the newt *Triturus*. By extirpating the brain and the spinal cord[8] it was concluded that Anurans (at least *Rana* and *Bufo*) did not have a vomiting centre in the central nervous system The present author, by using well-fed, healthy and live frogs (*Rana tigrina* and *Rana cyanophlyctis*) has observed that like mammals, these frogs also have a vomiting control centre in the medulla and that vomiting is not uncommon in frogs in the experimental and probably also in the natural environs.

Material and Method

Frogs collected locally were well-fed and were given subcutaneous injections of various concentrations of emetics. Out of the stimulants and emetics tried (nicotine, atropine, suprarenal extract, sodium hydrate, apomorphine, and tartar emetic) best results were obtained with apomorphine and tartar emetic[4–5]. Pithed and chloroformed frogs were dissected for intragastric injections of emetics and local applications of emetics on the wall of the stomach.

Observations and Conclusions

Thirty frogs were given subcutaneous injections through the dorsal lymph sinus and quickly left under glass bell jars for observations. Another 20 frogs were first narcotized with 1 ml of 1% urethan solution injected subcutaneously through the dorsal lymph sinus and dissected soon after. The stomach was split open. Emetics and stimulants were applied directly over the mucosa of the stomach with the help of cotton buds to observe peristalsis of the gastric muscles. Observations are recorded in Table I.

It may now be noted that vomiting was brought about by apomorphine and tartar emetic. Stimulants like suprarenal extract, atropine, nicotine and sodium hydrate could induce peristalsis only. Effects of mild and small doses of apomorphine and tartar emetic injections were amazingly spontaneous. The injected frogs became highly excited, they opened and closed their mouths in quick successions and the tongue was shot out quite frequently. Throat exhibited strong gulping movements apparently to swallow the vomitus back down to the stomach. These responses continued for about 8 minutes, after which they slowed down. In about 30 minutes from the time of injection the frogs became unconscious. It was also observed that large and strong doses of apomorphine and tartar emetic inhibited vomiting, probably causing paralysis of muscles[5,7] and in the dogs.

In frogs with damaged brains, no vomiting could be induced either by injecting the emetics or by direct application of emetics on the gastric mucosa, but peristalsis was produced by a similar treatment with

TABLE I

Vomiting and gastric peristalsis in frogs

Chemical	Concentration	Quantity injected or applied	Observations
Suprarenal extract	0·1%	0·5 ml	no response,
	0·3%	0·5 ml	slow peristalsis in stomach,
	0·5%	0·5 ml	peristalsis increased,
	1·0%	1·0 ml	peristalsis inhibited, no vomiting
Atropine	0·1%	0·5 ml	no response,
	0·3%	0·5 ml	slow relaxation of stomach muscles,
	0·5%	0·5 ml	relaxations advanced,
	1·0%	1·0 ml	relaxations advanced, no vomiting
Nicotine	0·1%	0·5 ml	immediate peristalsis,
	0·3%	0·5 ml	persitalsis advanced,
	1·0%	1·0 ml	peristalsis inhibited, no vomiting
Apomorphine	0·1%	0·5 ml	spontaneous reverse peristalsis in stomach and vomiting,
	0·3%	0·5 ml	peristalsis and vomiting,
	0·5%	0·5 ml	vomiting advanced,
	1·0%	1·5 ml	inhibitory effect on vomiting, muscles of the entire body paralysed in 15 minutes
Tartar emetic	0·1%	0·5 ml	slow reverse peristalsis and vomiting,
	0·3%	0·5 ml	vomiting advanced,
	0·5%	0·5 ml	vomiting advanced,
	1·0%	1·5 ml	all muscular activity paralysed in 12 minutes
Sodium hydrate	1 in 20,000 parts	0·1 ml	spontaneous contractions in the stomach, but no vomiting,
	do.	0·5 ml	all muscular activity paralysed

stimulants like sodium hydrate, nicotine and atropine. In frogs whose spinal cord alone was damaged, vomiting but not peristalsis, could be induced with the same chemicals. This was suggestive of the fact that reflexes could travel through the vagus nerve supplying the stomach while spinal nerves III, IV and V supplying the stomach were inactivated thus peristalsis was out of the question. Lastly, in frogs whose entire central nervous system was damaged no treatment of emetics or stimulants could induce either vomiting or peristalsis.

These experiments suggest a central control of vomiting somewhere in the medulla of the brain. Several workers[1,3,4,7] have demonstrated a vomiting centre and a chemoreceptor trigger zone in the medulla of the dog. A similar vomiting centre if not also a chemoreceptor trigger zone is suggested in the frogs used by this author.

The author is grateful to the management of Lucknow Christian College for all the encouragement.

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1. Borison, H. L. and Wang, S. C., *Pharmacol. Rev.*, 1953, **5**, 193.
2. — and —, *Arch. Neurol and Psychiat.*, 1950, **63**, 928.
3. Dixon, W. E., *Jour. Physiol., London*, 1902, p. 28
4. Eggleston, C. and Hatcher, R. A., *J. Pharmacol. and Expt. Therapy*, 1915, 7, 225.
5. Hatcher, R. A., *Physiol. Rev.*, 1924, **4**, 479.
6. — and Weiss, S., *J. Pharmacol. and Exptl. Therapy*, 1923, **22**, 139.
7. Klee, P., Der Brechakt, In *Handbuch der normalen and pathologischen physiologie*, A. Bethe, G. V. Bergmann, G. Embden, und A. Ellingen, Berlin, 1927, **8**, 441.
8. Leake, C. D., *J. Pharmacol. and Expt. Therapy*, 1923, **20**, 359.
9. Mellinger, C., *Arch. Ges. Physiol. Pfluegers*, 1881, **14**, 232.
10. Moore, J. A., *Physiology of the Amphibia*, Acad. Pr., N.Y., 1964.
11. Noble, G. K., *The Biology of the Amphibia*, Dover Publ., 1954
12. Nigam, H. C., *Zoology of Chordates*, 6th ed., S. Nagin and Co. Jullundur, India, 1977.
13. Thumas, L. J., *Arch. Pathol. Anat. Physiol.* 1891, p. 123.

PROLINE ACCUMULATION UNDER ZINC DEFICIENCY IN MUSTARD

A STRIKING accumulation of the imino acid proline occurs in many plants in response to water deficit[1,2] and salinity stress[3,4]. In the present paper it has been reported that there is accumulation of proline by deficiency of a micronutrient, Seeds of mustard (*Brassica juncea* L. var. T-59) were germinated in purified sand. After 10 days, uniform seedlings were selected and transplanted in ten litre glass troughs containing Hoagland's solution (half strength). Minor elements (except zinc) were added as suggested by Arnon[5]. There were three treatments (0, 0·01 and 0·05 ppm) of zinc. The chemicals used were of AR grade and purified by the method of precipitation and adsorption on phosphate and alkaline earth, autoclaving and thereafter extraction with dithizone[6]. Fifty seedlings were grown in each glass trough. Plants were supported in holes on a sun-mica lid with non-absorbent cotton wrapped around the stem. The aeration was done daily and the nutrient solution was replaced at weekly interval. Forty-five days after transplanting, plants were sampled, washed twice with double distilled water. The plants were separated into leaves, stem and root and dried in an oven at 60–70° C. Dry weight of leaves, stem and root per plant were recorded and the samples were analysed for zinc content by atomic absorption spectrophotometer. The proline content was analysed in leaves following the method of Bates, Waldren and Teare[7].

Plants grown in zinc deficient condition showed visual symptoms, about one month after transplanting. At 45 days, a marked decrease in dry weight of leaves, stem, root as well as tissue concentration and uptake of zinc was observed under zinc deficiency (Table I). The decrease in dry weight per plant was of the order of 60% and 51% as compared to plants grown in 0·01 and 0·05 ppm zinc levels respectively. Zinc level (0·01 ppm) in water culture appeared to be sufficient for growth of this mustard variety. The proline content in leaves increased under zinc deficiency (Table II). The accumulation of proline under zinc deficiency was about 4·7 and 8·0 times higher than that observed in plants grown in 0·01 and 0·05 ppm respectively.

TABLE I

Growth and zinc content in mustard var. T–59 45 days after transplanting

Zinc levels (ppm)	Leaves	Stem	Root	Total
		Dry weight (g/plant)		
0	0·281	0·399	0·110	0·790
0·01	0·616	1·098	0·268	1·982
0·05	0·548	0·895	0·171	1·614
LSD at 5% P	0·080	0·303	0·028	0·322
		Tissue concentration of zinc (ug/g dry wt.)		
0	19·62	13·16	31·40	
0·01	28·08	21·42	55·71	
0·05	31·90	23·66	73·91	
LSD at 5% P	4·86	5·63	7·49	
		Total content of zinc (ug/plant)		
0	5·51	5·24	3·45	14·21
0·01	15·39	23·52	14·92	53·83
0·05	19·64	21·17	12·63	53·45
LSD at 5% P	2·81	5·96	1·44	6·38

TABLE II

Proline content in leaves of mustard var. T 59, 45 days after transplanting

Zinc levels (ppm)	μ moles/g fr. wt.
0	9·21
0·01	1·95
0·05	1·15
LSD at 5% P	1·20

Accumulation of proline during both water and salinity stress has been suggested to be a consequence of reduction in cell osmotic potential[4]. Mg^{2+} or Ca^{2+} ions could also cause greater proline accumulation than iso-osmotic solutions of the non-ionic osmoticum[8]. It has not been possible to explain from the present study, how zinc deficiency caused the accumulation of proline. However, the growth inhibition under zinc deficiency could possibly cause the accumulation of other ions available in the nutrient solution and thus effect the proline accumulation either by changing the osmotic potential of the cell or by specific ion effect.

The accumulation of proline, independent of any effect on osmotic potential, may also not be ruled out as has also been postulated under conditions of low temperature stress[9].

A simple criterion for assessing the susceptibility of genotypes to zinc stress would be desirable in studies on zinc nutrition of crops. Whether, proline accumulation is sensitive enough to provide such a tool would be a matter of further investigation.

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1. Singh, T. N., Aspinall, D. and Paleg, L. G., *Nature New Biology*, 1972, **236**, 188.
2. —, Paleg, L. G. and Aspinall, D., *Aust. J. Boil. Sci.*, 1973, **26**, 45
3. Palfi, G. and Juhasz, J., *Acta Agron. Acad. Sci. Hung.*, 1970. **19**, 79,

4. Chu, T. M., Aspinall, D. and Paleg, L. G., *Aust, J. Plant Physiol.*, 1976, **3**, 219.
5. Arnon, D. I., *Am. J. Bot.*, 1938, **35**, 322.
6. Hewitt, E. J., *Sand and Water Culture Methods Used in the Study of Plant Nutrition*, Commonwealth Agricultural Bureaux, England, 1966.
7. Bates, L. S., Waldren, R. P. and Teare, I. D., *Plant and Soil*, 1973, **39**, 205.
8. Chu, T. M., Aspinall, D. and Paleg, D. G., *Aust. J. Plant Physiol*, 1976, **3**, 503.
9. —, — and —, *Ibid.*, 1974, **1**, 87.

REVIEWS AND ANNOUNCEMENTS

Reverse Osmosis and Synthetic Membranes. Edited by S. Sourira Jan. (National Research Council, Canada, Ottawa, KIA OR9), 1977. Pp. 598. Price $ 45.00.

Reverse Osmosis research of hardly 17 years has opened new horizons to unravel the mysteries surrounded with natural phenomenon such as Osmosis. Upto 1960, the scientific community believed Reverse Osmosis technique as academic pursuit. With the announcement of Loeb-Sourirajan membrane, completely new enthusiasm was evident in the scientific community for Reverse Osmosis field and as a corrollary, tremendous amount of scientific and technological activity followed. The literature in the form of research papers, reports, bulletins, journals, etc., continued to flow since then. There are several documents available giving accounts of Reverse Osmosis research at various institutions world over. It is to the credit of the whole scientific community that Reverse Osmosis technique has turned out to be a dependable unit operation for separation, concentration and fractionation of inorganic or organic substances in aqueous or non-aqueous solutions in the liquid or gaseous state. Thus, it earned the credit of being the versatile field of separation technology in the chemical process engineering.

The book entitled *Reverse Osmosis and Synthetic Membranes* under review is rich in contents and is a milestone in the history of literature brought out so far by any single document on the subject of Reverse Osmosis. The editor and the main contributor, Dr. S. Sourirajan, deserves all praise for his meticulously planning the book and presenting its contents in a systematic order. He has rightly said that the subject is evolving one, and since the literature is growing fast, in spite of its extensive coverage, the record imbibed in the book is by no means complete. But this shows his humbleness.

To measure and evaluate various approaches advanced for research in reverse osmosis technique where institutions and attitudes of scientists manifest in almost endless variety requires personal observation in depth and in greater detail. Exhaustive studies covered in the present book by Dr. Sourirajan are unique in nature and fulfil the requirements prescribed for understanding the science of reverse osmosis which has rightly been defined by the author as "A physico-chemical basis for Reverse Osmosis separation, material science of Reverse Osmosis membranes, and engineering science of Reverse Osmosis transport which together constitute the science of reverse osmosis". Besides his own contribution in the field of Reverse Osmosis, the valuable work done by his counterpart in other countries has been presented in the book with factual information. The selection of the material for the book has so ably been done that entire picture of RO research efforts is available to the reader.

Starting from Reverse Osmosis, a general separation technique, the book gives account of physico-chemical criteria for reverse osmosis separations, transport through reverse osmosis membranes, reverse osmosis process design, asymmetric cellulose acetate membranes, statistical design of experiments for optimizing the casting variables for cellulose acetate membranes, cellulose acetate and other cellulose ester membranes, grafted membranes for reverse osmosis, polyamide membranes, advances in development of sulphonated PPO and modified PPO membrane systems, PBI membranes, non-polysaccharide membranes, composite membranes, dynamic membranes, hollow fine fibre technology, spiral-wound reverse osmosis configuration, Westinghouse membrane techniques, British reverse osmosis techniques, and applications of reverse osmosis in the various fields such as food processing, pulp and paper industries, electroplating, treatment of domestic and municipal waters, gas permeation and separation by flow under pressure through microporous reverse osmosis membranes and tubular reverse osmosis plant design. Finally, Dr. Sourirajan summarises the accomplishments and prospects for reverse osmosis research in this book.

Each chapter and subject has its own merit in the area of Reverse Osmosis and it has been dealt in the most attractive, acceptable and simple form.

To find a researcher of the calibre of Dr. Sourirajan, will be itself an adventure into the trackless jungle of scholarship of the world. Devoted as he is, Dr. Sourirajan will continue to serve the Reverse Osmosis area by active research work and to provide such indispensable tools to the scientific community in general and reverse osmosis group of research workers in particular.

In my opinion this important book should find place in the shelves of the industry engaged in water treatment and scientists who are actively engaged in R & D work on reverse osmosis.

D. J. MEHTA.

Review on Indian Journal of Acarology, Vol. I (Nos. 1 and 2), 1976.

With thhe publication of Vol. I (Nos. 1 & 2) of *Indian Journal of Acarology* by the Acarological Society of India, the long-cherished aspiration and necessity of the Indian Acarologists for having exclusively their own journal have been fulfilled. Acarines represent one of the most major and important components of all terrestrial ecosystems and occupy a prominent position for their numerical abundance and wiversities not to speak of their economic importance. Considerable augmentation of researches on this group of arthropod in the universities and various research centres in India during the past decade demanded such an organ for quick publication of the results.

There are altogether eight taxonomic contributions in the first issue of the journal under discussion, from only four contributors. It is imperative, therefore, that all Indian Acarologists make it a point to contribute their papers to this journal for increasing its wealth and justifying the credibility as well for the publication of a separate journal. The aspects of results, publishable through this journal, remain amply wide. Papers on general soil biology, dealing with Acarina as one of the components of total biota, may hopefully find a place here. Thus, besides, fulfilling the need of Acarologists, it may also cater to the need of Soil Biologists for the publication of their results for which no exclusive journal is available in India.

It is desirable, however, to improve upon the get-up of the journal by raising the standard of printing, quality of illustrations (some of the diagrams used are substandard) and specially by improving the quality of block making. It is hoped that the journal in future would be able to publish papers embracing the bioecological aspects of Acarines for fulfilling its every objective.

Wide publicity from all quarters for encouraging the Acarologists and general soil biologists for publication of their results through this journal may go a long way for achieving its goal in near future.

The Chief Editor and members of the Executive Council of the Acarological Society of India deserve congratulations for bringing out this journal in time.

T. N. ANANTHAKRISHNAN.

Annual Review of Physiology, Vol. 39. (Annual Reviews Inc., 4139, El Caminoway, Palo Alto, California 94306), 1977. Pp. 640. Price: U.S.A. $ 17.00; Elsewhere $ 17.50.

The volume presents a series of reviews on advances in physiology, written by eminent scientists in their respective fields of specialization.

The prefatory chapter "My Scientific Odessay" by John C. Eccles, the Nobel award winner (1963), is an interesting autobiographical of his life and works. His realisation, after a long fruitful scientific career, that 'Scientists and Philosophers share the guilt of being dogmatic in promulgating claims, to a knowledge and understanding that devolves from their inflated self-esteem' is rather a sad reflection on the existing attitude of the Scientific community.

In the area of adaptation to environment, the topics reviewed are regulation of body fluids, cerebral mechanisms of importance in the control of body fluids, and role of physical activity in cardio-vascular health. The possibility of direct investigation of the molecular mechanisms of ion transport is the subject matter of 'Ion transport in reconstituted membranes'.

'Lymph formation and flow', 'Microcirculation' and 'Biophysical analyses of blood vessel walls and blood flow' review the recent advances in systemic circulation.

'Digestive enzymes of the pancreas', 'Gastrointestinal hormones' and 'Mechanism of hepatic bile formation' cover some facts of our recent knowledge on the digestive system.

'The Thyroid and its control', 'Regulatory peptides of the hypothalamus' and 'Peptide hormone receptors' present newer concepts on the wider physiological and pathological roles of the hormones.

Excitation-contraction coupling, operating in almost every aspect of cardiac physiology, new conceptual approach in cardiac dynamics, heterogeneity of nephron function, actions of hormones on the Kidney, Lipid and Lipid metabolism present the advancing knowledge in these specific areas.

"Osmoregulation in amphibians and reptiles" and "the interaction of body temperature and acid base balance in ectothermic vertebrates" are interesting chapters in the area of comparative physiology.

M. SIRSI.

Use of Non-human Primates in Biomedical Research. Edited by M. R. N. Prasad and T. C. Ananda Kumar. (Proceedings of an International Symposium held in New Delhi, India, November 3–8, 1975), 1977. Pp. xx + 426. Price : U.S. $ 40 ; India Rs. 300.

We are now on the frontier of many new discoveries in human biology, medicine and public health, when we urgently need new leads to meet the pressing medical and health problems of mankind. Biomedical scientists are increasingly aware of the vital rôle that non-human primates play in research development. Yet, it is a time when we face a declining supply of non-human primates which are so essential for research.

Recognizing the importance of non-human primates in the advancement of knowledge relevant to human health and welfare, the Indian National Science Academy organized an International Symposium on 'use of non-human primates in biomedical research', during November 3–8, 1975. This volume contains 37 papers presented at the Symposium.

The topics covered include (*i*) Non-human primates in the wild and their conservation ; (*ii*) Reproductive biology of non-human primates and their use in contraceptive technology and (*iii*) Use of non-human primates in Surgery, Neurobiology, Psychopharmacology, Nutrition, Immunology and Toxicological studies.

Focussing attention on the relevance of using non-human primate models in biomedical research, the steps that need to be taken to conserve the valuable natural resource of non-human primate populations and, other aspects, the symposium has emphasized the need for more extensive and intensive field programmes on a long range basis to achieve the objectives in view. The group of Indian Scientists participating in the symposium have suggested the establishment of a Primate Research Institute in India which could be the nucleus of an International Primate Research Centre.

Representing authoritative contributions of scientists from 14 nations, this volume will be a valuable reading for ecologists, wild life conservation and primate research workers and scientists engaged specially in biomedical research.

M. SIRSI.

INDIAN NATIONAL SCIENCE ACADEMY, NEW DELHI, 1977

At the Annual General Meeting of the Indian National Science Academy, held on Saturday, the 15th October 1977, Dr. R. Ramanna, Director, Bhabha Atomic Research Centre, Bombay, was re-elected President of the Indian National Science Academy.

The other Officers re-elected for the ensuing year are:

Vice-Presidents : Prof. R. C. Mehrotra, University of Delhi; Prof. A. K Sharma, Calcutta University; *Foreign Secretary* : Dr. A. R. Verma, National Physical Laboratory, New Delhi; *Secretaries* : Prof A. N. Mitra, University of Delhi; Dr. M. G. Deo, All India Institute of Medical Sciences, New Delhi; *Editors of Publications* : Prof. L. S. Kothari, Delhi; Prof. K. N. Saxena, Delhi.

Prof. B. Ramachandra Rao, Vice-Chairman, University Grants Commissions, New Delhi, was elected Treasurer.

The members of Council elected (or re-elected marked *). are:

Dr. F. Ahmad, Commissioner, Mines and Geology, Government of Jammu & Kashmir, Jammu/Srinagar; Dr. D. P. Antia, Flat No. 7, 10 Judges' Court Road, Alipore, Calcutta; *Prof. A. Bose, Kamdahari, Garia P.O., 24 Parganas, West Bengal; *Prof. J. Ganguly, Chairman, Dept. of Biochemistry and Head of Advanced Centre of Biochemistry, Indian Institute of Science, Bangalore; Dr. J. S. Kanwar, Associate Director, International Crops Research Institute for the Semi-Arid Tropics, Hyderabad; Dr. C. R. Krishna Murti, Deputy Director, Industrial Toxicology Research Centre, Lucknow; Prof. N. Balakrishnan Nair, Head, Dept. of Aquatic Biology & Fisheries and Dean, Faculty of Science, University of Kerala, Trivandrum. *Prof. M. S. Narasimhan, School of Mathematics, Tata Institute of Fundamental Research, Bombay; *Dr. Y. Nayudamma, Central Leather Research Institute, Adyar, Madras; Dr. A. S. Paintal, Director, V. P. Chest Institute, University of Delhi, Delhi-110007; *Dr. N. Parthasarathy, 16, Satyanarayana Avenue, Boat Club Road, Madras; *Prof. V. Puri, Professor

Emeritus, Institute of Advanced Studies, Meerut University, Meerut; *Prof. C. N. R. Rao, Professor, Solid State & Structural Chemistry Unit, Indian Institute of Science, Bangalore; *Prof. S. N. Sarkar, Head, Dept. of Applied Geology, Indian School of Mines, Dhanbad-826004; *Prof. G. P. Sharma, Head, Dept. of Zoology, Punjab University, Chandigarh; *Dr. K. Sundaram, Director, Biomedical Group, Bhabha Atomic Research Centre, Bombay; *Prof. S. K. Trehan, Professor of Applied Mathematics, Punjab University, Chandigarh; Dr. H. V. K. Udupa, Director, Central Electro-Chemical Research Institute, Karaikudi.

The Asiatic Society, Calcutta, has renominated Prof. S. Deb, FNA, Formerly Professor and Head, Geology, Department, Jadavpur University, Calcutta.

The National Academy of Sciences, India, Allahabad has nominated Dr. B. D. Nagchaudhuri, President of that Academy, as their representative.

The Indian Science Congress Association, Calcutta, will be represented by its General President, Dr. S. M. Sircar.

Dr. A. Ramachandran, FNA, Secretary, Department of Science and Technology, continues to be the representative of the Government of India.

Dr. B. P. Pal, immediate Past President of the Academy, is also a member of the Council.

FELLOWS ELECTED AT THE ANNUAL GENERAL MEETING

Dhawan, S., Director, Indian Institute of Science, Bangalore; Agarwala, S. C., Professor of Botany, Lucknow University; Biswas A. B., Department of Chemistry, Indian Institute of Technology, Bombay; Biswas, B. B., Professor of Plant Biochemistry, Bose Institute, Calcutta; Bhimasankaram, V. L. S., Professor of Exploration Geophysics, Osmania University, Hyderabad; Chandrasekhar, S., Liquid Crystal Laboratory, Raman Research Institute, Bangalore; Chidambaram, R., Head, Neutron Physics Section, Bhabha Atomic Research Centre, Bombay; Chopra, K. L., Professor, Indian Institute of Technology, New Delhi; Choudhury, B., Division of Vegetable Crops & Floriculture, Indian Agricultural Research Institute, New Delhi; Ghosh, Asok, Professor of Zoology, Calcutta University, Calcutta; Jain, P. C., Professor of Mathematics, Indian Institute of Technology, Bombay; Khush, G. S., International Rice Research Institute, Manila; Maheshwari, S. C., Professor of Botany, University of Delhi, Delhi; Mohan Ram, H. Y., Professor of Botany, University of Delhi, Delhi; Nag, B. R., Institute of Radio Physics & Electronics, Calcutta; Nasipuri, D., Professor of Chemistry, Indian Institute of Technology, Kharagpur; Patel, C. C., Professor of Inorganic & Physical Chemistry, Indian Institute of Science, Bangalore; Pisharoty, P. R., Professor, Physical Research Laboratory, Ahmedabad; Prem Narain, Professor, Institute of Agricultural Research Statistics, New Delhi; Radhakrishnan, A. N., Professor of Biochemistry, School of Life Sciences, Hyderabad University, Hyderabad; Raghavan, S., Associate Professor, Tata Institute of Fundamental Research, Bombay; Ram Das, V. S., Professor and Head, Department of Botany, Sri Venkateswara University, Tirupati; Rajasekharasetty, M. R., Senior Professor and Head, Department of Post Graduate Studies & Research in Zoology, University of Mysore, Mysore; Sharma, K. N., Chairman & Professor of Physiology, University College of Medicine, New Delhi; Sinha, A. P. B., Deputy Director, National Chemical Laboratory, Poona; Sodha, M. S., Professor of Physics, Indian Institute of Technology, New Delhi; Subba Rao, E. C., Professor, Department of Metallurigical Engineering, Indian Institute of Technology, Kanpur; Sukheshwala, R. N., Professor of Geology, St. Xavier's College, Bombay; Swaminathan, S., Department of Organic Chemistry, Madras University, Madras; Thapliyal, J. P., Professor of Zoology, Banaras Hindu University, Varanasi.

FOREIGN FELLOWS ELECTED

Professor Sir Barton, Derek (Harold Richard), London; Professor Blamont, Jacques-Emile, Paris; Professor Brown, Herbert, C., Indiana, U.S.A.; Professor Ochoa Severo, New Jersey, U.S.A.; Dr. Tata, Jamshed R., London.

SCIENCE ACADEMY MEDALS FOR YOUNG SCIENTISTS FOR THE YEAR 1977 AWARDED TO:

Dr. Ateeq Ahmad, Central Drug Research Institute, Lucknow; Dr. P. Balaram, Indian Institute of Science, Bangalore; Dr. Asha Chandola, Banaras Hindu University, Varanasi; Dr. S. K. Dattagupta, Reactor Research Centre, Kalpakkam; Dr. S. K. Dubey, All India Institute of Medical Sciences, New Delhi; Mrs. V. P. Joshi, Bhabha Atomic Research Centre, Bombay; Dr. S. C. Kak, Indian Institute of Technology, New Delhi; Dr. A. S. Raghavendra, Sri Venkateswara University, Tirupati; Dr. R. K. S. Rathore, Indian Institute of Technology, Kanpur; Dr. N. M. Singhi, Tata Institute of Fundamental Research, Bombay; Dr. H. Sinvhal, University of Roorkee, Roorkee; Shri A. K. Suri, Bhabha Atomic Research Centre, Bombay; Dr. R. C. Sobti, Punjab University, Chandigarh; Shri Vijay Kumar, Atomic Research Centre, Modular Laboratory, Bombay.

FOURTH ANNUAL CONVENTION OF THE ASSOCIATION OF EXPLORATION GEOPHYSICISTS, INDIA

The Fourth Annual Convention of the Association of Exploration Geophysicists is scheduled to be held from 19th to 21st January, 1978 at Andhra University, Waltair, Andhra Pradesh, India, during which a three day seminar on 'Exploration Geophysics' will be held on the following themes: (*i*) Geophysical technology; (*ii*) Instrumentation; (*iii*) Geophysical data processing; (*iv*) Geological mapping; (*v*) Exploration for ore deposits, oil, natural gas and other energy resources, and ground water and (*vi*) Engineering geophysics. Special Theme: 'Ocean Exploration'.

Papers are invited for presentation at the above seminar. Abstracts of papers (200–300 words) may be sent before 15th December, 1977 and full papers before 31st December, 1977 to Dr. V. L. S. Bhimasankaram, Centre for Geophysics, Osmania University, Hyderabad 500 007.

For details please contact Dr. V. L. S. Bhimasankaram, Centre for Geophysics, Osmania University, Hyderabad.

AWARD OF RESEARCH DEGREES

Bangalore University has awarded the Ph.D. degree in Geology to Shri R. Nijagunappa.

Bhagalpur University has awarded the Ph.D. degree in Botany to Shri A. K. Roy.

Berhampur University has awarded the Ph.D. degree in Chemistry to Sri K. C. Samantra.

Karnatak University, Dharwar, has awarded the Ph.D. degree in Zoology to Shri Ravindra Paul and to Shri Mallikarjun Annappa Hooli; Ph.D. degree in Geology to Shri Vishwanath Basavantappa Koppad.

Osmania University, Hyderabad, has awarded the Ph.D. degree in Geology to Shri Vinnakota Venkata Rama Sarma and Shri Gopikishan.

University of Cochin has awarded the Ph.D. degree in Marine Sciences to Shri C. J. Cherian.

Utkal University, Bhubaneswar, has awarded the Ph.D. degree in Botany to Shri K. L. Hakim and Smt. Aruna Kumari Misra; Ph.D. degree in Chemistry to Shri Hemantha Kumar Pratihari and to Shri Nirod Kumar Mohanty.

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degree in Chemistry to Shri G. Nagaraj; Ph.D. degree in Mathematics to Shri L. Nagamuni Reddy.

Tamil Nadu Agricultural University, Coimbatore, has awarded the Ph.D. degree in Agriculture to: Shri P. V. Marappan, Shri V. Ramaswamy, Shri S. Krishnaswami and Shri R. Arumugam.

The M.S. University of Baroda has awarded the Ph.D. degree in Physics to Shri Vinay Baburao Kamble and to Shri Subhashchandra Ratilal Pandya; Ph.D. degree in Chemistry to Shri Ashabhai Parsotambhai Prajapati; Ph.D. degree in Geology to Shri Ravindra Nath Pande; Ph.D. degree in Zoology to Shri Sumanbhai Thakorbhai Patel.

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MANGANESE(III) COMPLEXES OF DITHIOCARBAMATES DERIVED FROM SOME HETEROCYCLIC SECONDARY AMINES

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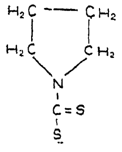
ABSTRACT

Manganese(III) complexes of dithiocarbamates derived from heterocyclic secondary amines, pyrrolidine, piperidine and tetrahydroisoquinoline have been prepared. They are of the general formula, MnL_3 where L is a ligand. The complexes are non-electrolytes in nitrobenzene and acetonitrile. Magnetic moment data show that they are of the spin-free type. Infrared spectra reveal the bidentate nature of the ligands. The electronic spectra suggest a lowering of symmetry from O_h.

DIALKYL dithiocarbamate complexes have been the subject of numerous investigations[1-4]. Comparatively, very little work has been done on the complexes of the dithiocarbamates derived from heterocyclic secondary amines. The results of our studies on the complexes of manganese(III) with pyrrolydyl-N-carbodithioate(I), piperidyl-N-carbodithioate(II) and tetrahydroisoquinoline-N-carbodithioate(III) are reported.



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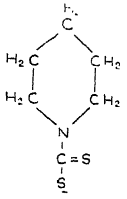

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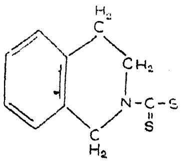

III

Sodium salts of the N-carbodithioates were prepared by the following method. An aqueous solution (200 ml. 5N) of sodium hydroxide and 0·5 mole of the appropriate amine were introduced into a three necked flask, cooled in a freezing mixture of ice and salt. Carbon disulphide (32 ml) was added dropwise from the separating funnel and the mixture was stirred for about 2 hr. The solid that separated out was washed several times with petroleum ether and recrystallised from water.

The complexes were prepared by mixing methanolic solutions of stoichiometric amounts (metal : ligand as 1 : 3) of manganese(III) acetate dihydrate and respective sodium-N-carbodithioates with stirring, when brown crystals of the complexes separated. The crystals were filtered, washed with methanol and dried in vacuum.

All the complexes are brown in colour and were found to be non-hygroscopic. They are soluble in benzene, CCl_4 and acetonitrile but are insoluble or only sparingly soluble in water and methanol.

Manganese in the complexes was estimated as pyrophosphate. Sulphur was estimated as $BaSO_4$ after bromine oxidation of the complexes.

The analytical data given in Table I show that the complexes have the general formula MnL_3 where L is a ligand with a uninegative charge. Conductivity measurements were made on a Philips GM 4249 conductivity bridge. The conductance data show the non-electrolytic nature of the complexes both in acetonitrile and nitrobenzene. The magnetic measurements were made at room temperature using the Gouy method. The effective magnetic moment values (Table I) show that the complexes are of the spin-free type. These further indicate that manganese in the complexes are in the trivalent state with four unpaired electrons. The complexes do not show any magnetic interaction.

Usually, dithiocarbamates behave as monovalent bidentate ligands. Unidentate behaviour has been reported in the case of ruthenium(III) complex[5] where a number of additional bands at 970–980, 1060–1065, 1260–1265, 1410 and 1460–1470 cm^{-1} are observed. The infrared spectra of the complexes under investigation resemble those of the bidentate dithiocarbamates. A strong band observed around 1500 cm^{-1} is assignable to the partially π-bonded C–N stretch[6]. In sodium tetrahydroisoquinolinyl-N-carbodithioate, the band assignable to the C–N stretch occurs at 1440 cm^{-1}. In the case of the pyrrolidyl and piperidyl derivatives, the C–N stretch occurs at 1460 and 1470 cm^{-1} respectively. The magnitude of C–N stretch depends on the ability of the substituent on the nitrogen atom to donate electrons. The present studies show that the heterocyclic ring substituents on the nitrogen are in general less electron donating to the C–N bond as compared to the alkyl substituents. The substituent effect is quite marked in the case of the tetrahydroisoquinolinyl derivative. The

TABLE I

Analytical and physical data

Complex	Manganese % Found	Sulphur % Found	Nitrogen % Found	μ_{eff} B.M.	Molar conductance λ_m ohm^{-1} cm^2 mole^{-1}		IR frequencies and their assignments	
					Nitrobenzene	Acetonitrile	Frequency cm^{-1}	Assignments
Sodium tetrahydroisoquinolinyl-N-carbodithioate	..	..	..	..	..	..	1440 s 960 m	ν_{C-N} ν_{C-S}
Tris (tetrahydroisoquinolinyl-N-carbodithioate) Mn(III)	7·86 (8·02)	28·09 (28·02)	6·05 (6·13)	4·97	0·2	23·1	1480 s 965 m	ν_{C-N} ν_{C-S}
Sodium pyrrolydyl-N-carbodithioate	..	..	..	..	..	..	1460 m 945 s	ν_{C-N} ν_{C-S}
Tris (pyrrolydyl-N-carbodithioato) Mn(III)	10·60 (11·14)	39·02 (38·95)	8·48 (8·52)	4·87	0·2	26·9	1470 vs 960 m	ν_{C-N} ν_{C-S}
Sodium piperidyl-N-carbodithioate	..	..	..	..	..	..	1470 s 970 s	ν_{C-N} ν_{C-S}
Tris (piperidyl-N-carbodithioto) Mn(III)	9·60 (10·27)	36·04 (35·90)	7·64 (7·85)	5·07	0·1	18·2	1480 vs 960 s	ν_{C-N} ν_{C-S}

Calculated values are given in parenthesis.

C–S stretching frequencies for the tetrahydroisoquinolinyl, piperidyl and pyrrolydyl derivatives of manganese(III) are located at 965, 960 and 960 cm^{-1} respectively. The complex with lowest ν_{asy} C–S is the most stable. On this basis, the three complexes under investigation have comparable stability.

The electronic spectra (chloroform) show bands around 28000 cm^{-1} assignable to charge-transfer processes[7]. The spectra also show bands around 20000 cm^{-1} and 16000 cm^{-1} assignable to the *d–d* transitions. Usually, octahedral manganese(III) complexes show only one band assignable to $^5E_g \rightarrow {}^5T_{2g}$. The occurrence of more than one band in the present complexes show a lowering of symmetry from O_h. The tris chelate complexes of the type under study will most probably belong to the point group D_3 because of the disymmetry caused by the chelating agent. In Mn(III) complexes, in addition to this, strong Jahn–Teller distortion is expected to be operative and hence the present complexes may be considered to have a symmetry C_2. The bands observed in the present complexes can be assigned to transition involving the split components of ^5E_g and $^5T_{2g}$ in a low symmetry ligand field.

A thermogravimetric analysis of the complexes show that the complexes are stable upto 200° C, then the decomposition occurs in one stage.

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1. White, A. H., Roper, R., Kokot, E., Waterman, H. and Martin, R. L., *Aust. J. Chem.*, 1964, 17, 295.
2. Martin, R. L. and White, A. H., *Inorg. Chem.*, 1967, 6, 712.
3. Nair, C. G. R. and Yusuff, K. K. M., *Inorg. Nucl. Chem. Lett.*, 1975, 11, 753.
4. Prabhakaran, C. P. and Patel, C. C., *Indian J. Chem.*, 1969, 7, 1257.
5. Dominicano, A., Vaciago, A., Zambonelli, L., Loader, P. L. and Venanzi, L. M., *Chem. Commun.*, 1966, p. 476.
6. Chatt, J., Duncanson, L. A. and Venanzi, L. M., *Acta Chem. Fenn.*, 1956, 29 B, 75.
7. JØgensen, C. K., *Acta Chem. Scand.*, 1962, 16, 2406.

APPLICATION OF REGRESSION ANALYSIS TO HYPOTENSIVE ACTIVITIES OF PYRIDAZINONES

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ABSTRACT

Regression analysis of 32 compounds in pyridazinone series has been performed using Free-Wilson method. Individual group contributions for 28 analogues have been calculated separately by taking rank order and logarithm of hypotensive activities (MABP) as dependent variables. The degree of agreement between the observed and the calculated activities is estimated at 92% variance level with F = 10·614 and multiple correlation coefficient, $r = 0{\cdot}986$. Based on the group contributions and on the number of times a substituent occurs at a particular position in the compound, a quantitative structure-activity relationship has been studied.

INTEREST in the design of biologically active compounds[1] using quantitative structure-activity relationship (QSAR) models has been mainly from our observation that out of a number of 2-substituted thiazolidines synthesized and tested for anti-radiation activity, only 'one' compound showed good activity and the remaining no activity[2]. In order to predict the activity for additional compounds in this series, application of Free-Wilson method[3] and molecular orbital method of complete neglect of differential overlap (CNDO) has been made[4]. Recently some compounds for antihypertensive activity in pyridazine and phthalazine series have been studied by calculating charge densities on essential atoms and by the molecular conformation of substituents using CNDO and extended Huckel theory (EHT)[5]. Since the compounds predicted for activity in the above series showed interesting biological activity on their actual synthesis, it is attempted to study QSAR of the present system, 6-phenyl substituted-4, 5-dihydro-3(2H)-pyridazinones (Fig. 1) for hypotensive activity. In this study, the method of regression analysis using additivity model of Free-Wilson[6] has been made.

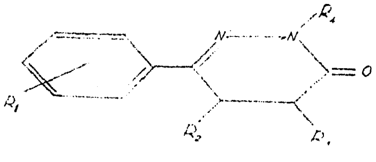

FIG. 1

The Free-Wilson method which has been applied to number of drug molecules[3,7,8] for the purpose of predicting therapeutically active molecules, is based upon an additive mathematical model. This is presented in the form of a linear equation (1).

$$\text{Biological response} = \mu + \Sigma \text{ group contributions} \quad (1)$$

where μ represents overall average activity of the parent molecule. The parameter for the biological response may be expressed in terms of log (1/C) where C is the molar concentration of the drug, per cent inhibition of enzyme PI_{50} or per cent survival, etc. In the present analysis, the hypotensive activity of dihydropyridazinones has been considered in terms of rank order and in terms of logarithm of mean arterial blood pressure (MABP) of normotensive rats 4 hours after a single oral dose of 100 mg/kg. The activity data have been taken from Curran and Ross[9].

The compounds considered in the analysis are shown in Table I. It consists of activity data for 28 analogues of 32 compounds written in the form of structural matrix. The occurrence of groups in a particular compound is indicated by a numeral (or coefficient). The analysis was carried out by considering one dependent variable at each substituent position and then converting the matrix to a contracted matrix. In this system, m-CH_3CONH at R_1, CH_3 at R_2 and R_3 and C_6H_5 CH_2CH_2– at R_4 were considered as dependent variables. This gives 24 analogues of dihydropyridazinones consisting 32 compounds (equations). These equations were solved separately by taking rank order and logarithm of hypotensive activities (MABP) as dependent variables (observed activity) and coefficients as independed variables on a computer (DEC–10) using the least square method.

RESULTS AND DISCUSSION

By applying regression analysis, the values for regression constant and group contributions to the hypotensive activity were obtained (Table II). These have been calculated separately by taking rank order [blood pressure (mm). 65–75 = 4, 76–85 = 3, 86–95 = 2, 96–105 = 1 and > 106 = 0] and logarithm of hypotensive activities (MABP) of pyridazinones. The criteria for activity are that MABP must be $\leqslant 90$ for treated and 120 for untreated animals. By substituting the values of μ and group contributions into eq. (1), the activities have been calculated and are reported in terms of fall or rise of blood pressure (Table I). It is clear from these results that a good agreement

TABLE I

*Observed and calculated hypotensive activities of dihydropyridazinones**

Compound No.	Substituents				Activity (MABP) obsd.	Activity (MABP) calcd.
1	A	T	V	X	90	90
2	B	T	V	X	98	99
3	C	T	V	X	116	116
4	D	T	V	Y	110	110
5	E	T	V	Y	115	113
6	E	T	V	Z	112	112
7	E	T	V	AA	110	110
8	E	T	V	AB	122	122
9	E	T	W	X	106	113
10	F	T	V	X	82	83
11	G	T	V	X	132	132
12	E	U	V	X	85	83
13	H	T	V	X	114	114
14	I	T	V	X	52	87
15	J	T	V	X	98	98
16	K	T	V	X	102	102
17	L	T	W	X	100	101
18	M	T	V	X	98	98
19	N	T	W	X	102	97
20	F	T	W	X	90	89
21	N	U	V	X	68	72
22	L	U	V	X	73	75
23	B	U	V	X	80	79
24	F	U	V	X	70	66
25	F	T	V	Y	84	90
26	O	U	V	X	86	86
27	L	U	V	Y	85	82
28	P	T	V	X	107	107
29	Q	T	V	X	112	112
30	R	T	W	X	103	103
31	S	U	V	X	82	82
32	G	U	V	X	66	70

*Alphabets indicate the occurrence of substituents as given in Table II. A structural matrix can be constructed by taking substituents A to S at R_1, T and U at R_2, V and W at R_3 and X to AB at R_4. For details see refs. 3 and 7.

TABLE II

Substituent contribution to hypotensive activity of dihydropyridazinones

No.		Substituents	No. of times occurring	Group Contribution by Rank order	Group Contribution by Logarithm
R_1 =	A	p-F	1	0·604	−0·038
	B	m-NO_2	2	−0·286	0·003
	C	p-OH	1	−1·396	0·074
	D	p-Br	1	−0·801	0·014
	E	p-Cl	6	−0·208	0·023
	F	p-CN	4	1·495	−0·076
	G	p-Cl. C_6H_4- P-C_6H_4	1	−1·396	0·129
	H	p-COOH	1	−1·396	0·066
	I	m-CN	2	0·715	−0·052
	J	p-$(CH_3)_2$ $N\text{-}\overset{\overset{O}{\Vert}}{C}\text{-}S$	1	−0·396	−0·001
	K	p-$(CH_3)_2$ $N\text{-}\overset{\overset{S}{\Vert}}{C}\text{-}O$	1	−0·396	0·018
	L	p-CH_3CONH	3	0·533	−0·021
	M	p-$CONH_2$	1	−0·396	−0·001
	N	p-NH_2	2	0·589	−0·039
	O	m-NH_2	1	−1·175	−0·039
	P	p-C_6H_4	1	−0·396	0·037
	Q	p-$CH_3O.C_6H_4$	1	−0·396	0·358
	R	H,	1	0·352	−0·012
	S	m-CH_3CONH	1	−0·175	0·019
R_2 =	T	H	23	−0·450	0·027
	U	CH_3	9	1·281	−0·069
R_3 =	V	H	27	0·117	−0·005
	W	CH_3	5	−0·632	0·028
R_4 =	X	H	25	0·198	−0·009
	Y	CH_3	4	−0·398	0·027
	Z	p-Cl. C_6H_4	1	−0·991	0·026
	AA	C_6H_5	1	−0·991	0·019
	AB	C_6H_5-CH_2-CH_2	1	−1·374	0·086

xists between the calculated and observed ctivities. The multiple correlation coefficient s 0·986 and F is 10·614. The ratio between the ariance of the estimated activity and the variance f the observed activity is found at 92% level.

By comparing the individual group contributions alculated as above, it can be observed that the roup contributions obtained by using rank order f activities are more significant and suggestive for ructure-activity correlations than using logarithms f activities. Based on these group contributions nd on the number of times a particular sub-ituent occurs at a particular position in the olecule, it is possible to assess the ability of ese substituents for activity. At position R_1 of ihydropyridazinones, out of 19 substituents which nclude both electron-withdrawing and electron-eleasing groups, only 6 substituents have positive roup contributions. These are in the order : -CN > *m*-CN > *p*-F and *p*-NH_2 > *p*-CH_3CONH > H respectively. *p*-chloro group occurring once ith a group at R_3 and four times with different roups at R_4 did not contribute to any activity. owever, this substituent along with methyl roup at R_2 gave better activity. *P*-cyano nd *m*-cyano groups which occur along with roups at R_2, R_3 and R_4 showed interesting ctivity. The methyl groups at R_2 position show ositive group contribution and produced active ompounds while those at R_3 produced no effect. one of substituents occurring at R_4 position gave ny activity and these groups showed negative alues of group contributions. From this analysis, becomes apparent that groups at R_3 and R_4 do not contribute to the activity and hence produce inactive compounds. This explanation on structure-activity analysis can further be supported by the results of our earlier studies[5] on pyridazine and phthalazine derivatives that nitrogen atoms in these molecules must be free for biological response. It may be assumed in the pyridazinone system that unsubstituted nitrogen atoms favour the activity.

The findings are being explored for the synthesis of compounds for which activity has been predicted.

ACKNOWLEDGEMENT

The financial assistance from the Bombay University is gratefully acknowledged. I thank Dr. J. K. Lalla, Principal of this college, for his encouragement.

1. Kulkarni, V. M. and Govil, G., *J. Sci. Ind. Res.* (In press).
2. — and Tipnis, H. P., *Curr. Sci.*, 1972, **41**, 637.
3. —, *Indian J. Chem.*, 1976, **14 B**, 190.
4. Tipnis, H. P. and Kulkarni, V. M., In : *Quantitative Structure–Activity Relationships*, Edited by M. Tichy (Akademiai, Budapest), 1973, p. 171.
5. Kulkarni, V. M. *Indian J. Biochem. Biophys.*, 1976, **14**, 362.
6. Free (Jr.), S. M. and Wilson, J. W., *J. Med. Chem.*, 1964, **7**, 395.
7. Thomas, J., Berkoff, C. E., Flagg, W. B., Gallo, J. J., Haff, R. F., Pinto, C. A., Pellerano, C. and Savini, L., *Ibid.*, 1975, **18**, 245.
8. Schaad, L. J., Werner, R. H., Dillon, L., Field, L. and Tata, C. M., *Ibid.*, 1975, **18**, 344.
9. Curran, M. V. and Ross, A., *Ibid.*, 1974, **17**, 273.

SULPHENYL DERIVATIVES OF ORTHO-MERCAPTO AZO COMPOUNDS: SYNTHESIS OF 4-DIMETHYLAMINO-AZOBENZENE-2′-SULPHENYL BROMIDE—ITS U.V. AND VISIBLE SPECTRA

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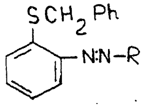
ABSTRACT

With a view to synthesizing new sulphenyl bromides of ortho-mercapto-azo-compounds, the debenzylation reactions of 2-arylazo-1-phenyl benzyl sulphides (I, *a*, *c*, *d*) and 2-arylazo-1-naphthyl benzyl sulphides (II) are carried out. Only (I *d*) yields the sulphenyl bromide (I *e*), all other compounds are converted into their respective disulphides [(III *a*, *b*) and (IV)]. The U.V. and visible spectra of (I *e*) are discussed.

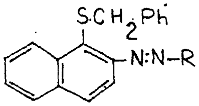
HOUGH Specklin and Meybeck[1] failed to diazotise *o*-benzyl thioaniline under a variety of conditions, urawoy *et al.*[2] could diazotise the same with nitrosyl lphuric acid and conc. sulphuric acid at 0° C and upled this diazonium compound successfully with 2-naphthol to get (I *a*). They were unable to debenzylate (I *a*), to sulphenyl bromide but obtained the disulphide (III *a*) instead.

So far no sulphenyl halide of ortho-mercapto azo compound is known to contain any electron donating groups like phenolic-hydroxy, amino, substituted amino or phenolic-ether group, in their ring systems. With a view to synthesizing such compounds, we diazotised 2-benzylthio-aniline and 1-benzylthio-2-naphthylamine with nitrous acid in ethanol medium. Though these diazonium compounds proved to be stable for 5–15 secs, they could be effectively coupled with a number of active substrates like 2-naphthol, phenol, *p*-cresol and dimethyl aniline, under alkaline conditions below 0° C, to give the expected compounds (I *a*, *b*, *c*, *d* and II)



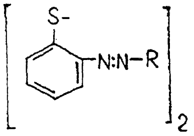
(I)

a. R = 2-hydroxy-1-naphthyl
b. R = *p*-hydroxy phenyl
c. R = 5-methyl-2-hydroxy phenyl
d. R = *p*-dimethyl amino phenyl
e. $\left\{ \begin{array}{l} -SCH_2Ph = -SBr \\ R = p\text{-dimethyl amino phenyl} \end{array} \right.$



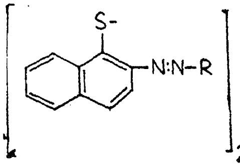
(II)

R = 2-hydroxy-1-naphthyl



(III)

a. R = 2-hydroxy-1-naphthyl
c. R = 5-methyl-2-hydroxy phenyl



(IV)

R = 2 hydroxy-1-naphthyl

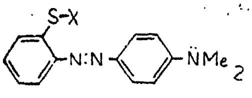
Attempted debenzylation of (I *a*, *c*) and (II *a*) by bromination in hot acetic acid or in warm or cold carbon tetrachloride (both in presence and in absence of iodine catalyst) yielded no sulphenyl bromide but only the corresponding disulphides (III *a*, *c*) and (IV).

On the other hand 2′-benzylthio-4-dimethylamine-azobenzene (I *d*) could successfully be converted to the sulphenyl bromide (I *e*) by treating it with bromine in carbontetrachloride at 40° C; debenzylation proceeded faster in the presence of iodine. But debenzylation reaction failed with bromine in hot acetic acid. This sulphenyl bromide was found to be stable towards water and alcohol and could be recrystallised unchanged from aqueous alcohol. Like other sulphenyl bromides of the series, it also underwent double decomposition with potassium iodide. Preliminary investigations show that this compound is an effective electrophile.

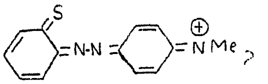
U.V. and visible spectra of 4-dimethylamino-azobenzene-2′-sulphenyl bromide:

The U.V. and visible spectra of this compound in ethanol show three bands — one high intensity band at 470 nm and two low intensity bands at 560 nm and 375 nm; whereas, in solvents benzene, chloroform and water, only two bands are shown, one high intensity band around 550 nm and a low intensity band around 375 nm. The band at 375 nm is a $\pi \rightarrow \pi^*$ band, the band around 550 nm may be due to a "Hydrazone" structure (VI) of the compound in the solvents. The band at 470 nm in ethanol solution is perhaps due to the "azo-structure" (V) of the compound formed due to partial solvolysis. This is supported by the fact that the 470 nm band disappears when the spectrum of the ethanolic solution of the sulphenyl bromide was taken in presence of added bromide ions. In ethanol, therefore, the sulphenyl bromide remains as a mixture of the "azo-structure" with a small amount of "hydrazone structure".

In sulphuric acid solution, however, only the band at 350 nm is obtained. Both the "azo-structure" and the "hydrazone structure" are not formed due to protonation of $-\ddot{N}Me_2$ group and the $-\ddot{N}=\ddot{N}-$ group.



(V)



(VI)

EXPERIMENTAL

A. *Diazotisation of* o-*benzylthioaniline and coupling of the diazonium compound with 2-naphthol*:

o-Benzylthioaniline (2 g) dissolved in ethanol (10 ml) at 0° C was mixed with sodium nitrite (0·5 g) in water (10 ml) at −5° C. Then hydrochloric acid (5 ml, 4 N) was added and the resulting diazonium chloride solution was immediately poured into a well stirred ice cooled solution of 2-naphthol (1·5 g) in sodium hydroxide (20 ml, 20%) at a time. The stirring was continued for 30 minutes, at −3° C. The resulting red precipitate of 2-(2′-hydroxy-1′-naphthyl-azo-)-phenyl benzyl sulphide (I *a*) was filtered off, washed and dried (1·95 g, 53% yield) gave brown red crystals from ethanol.

m.p. 161°–162° C.

$C_{23}H_{18}N_2SO$ requires C = 74·59%, H = 4·86%, N = 7·56%.
Found C = 74·42%, H = 4·74%, N = 7·49%.

The compound (I *a*) was identical with the compound obtained by the condensation of *o*-benzylthioaniline with 1-nitroso-2-naphthol.

B. The coupling reactions of diazotised *o*-benzylthioaniline with phenol and *p*-cresol and that of diazotised 1-benzylthio-2-naphthyl amine with 2-naphthol were carried out similarly as under A, to get the compounds 2-(*p*-hydroxy phenyl)-phenyl benzyl sulphide (I *b*), 2-(5-methyl-2-hydroxy phenyl)-phenyl benzyl sulphide (I *c*) and 2-hydroxy-1′-benzylthio-2′, 1-azo naphthalene (II) respectively. All of them were crystallised from hot ethanol.

I. (*b*) Crystallised as orange red crystals. m.p. = 143°–144° C, yield = 45%.

$C_{19}H_{16}N_2SO$ requires C = 71·25%, H = 5·06%, N = 8·75%.
Found C = 70·98%, H = 5·31%, N = 8·24%.

I. (*c*) Crystallised as red plates. m.p. = 102°– 103° C. yield = 51%.

$C_{20}H_{18}N_2SO$ requires C = 71·82%, H = 5·38%, N = 8·38%.
Found C = 71·20%, H = 5·33%, N = 8·51%.

II. Crystallised as red needles with a greenish lustre m.p. = 204°–205° C, yield = 57%.

$C_{27}H_{20}N_2SO$ requires C = 77·14%, H = 4·76%, N = 6·66%.
Found C = 77·56%, H = 4·37%, N = 6·83%.

C. 4-*Dimethylamino-2′-benzylthio-azobenzene* (I *d*) :

The diazonium solution prepared as under A was immediately added to a solution of dimethyl aniline (3 ml) in ethanol (10 ml) kept at – 5° C and the resulting solution was quickly made alkaline with sodium acetate solution (10 ml, 20% solution). A red precipitate formed at once, filtered and dried (1·4 g, 40% yield). It gave orange red crystals from hot ethanol, m.p. = 137°–139° C.

$C_{21}H_{21}N_3S$ requires C = 72·62%, H = 6·05%, N = 12·1%.
Found C = 72·45%, H = 5·92%, N = 11·85%.

D. *Attempted debenzylation of* (I *a, c*) *and* (II) :

(*a*) *In hot acetic acid.*—I *a* (1·85 g) in glacial acetic acid (10 ml) was refluxed. To it was added a solution of bromine (0·8 g) in acetic acid (5 ml) through the condenser. The mixture was refluxed (10 minutes), the solution was cooled. Red crystals of III *a* filtered off, washed and dried (1·23 g), gave red crystals from ethanolic benzene, m.p. = 182°–183° C.

$C_{32}H_{22}N_4S_2O_2$ requires C = 67·02%, H = 3·94%, N = 5·73%.
Found C = 66·98%, H = 4·12%, N = 5·78%.

(*b*) *In carbon tetrachloride.*—To a solution of I,*a* (1·85 g) in carbon tetrachloride (10 ml) kept at 40° C was added a solution of bromine (0·8 g) in carbon tetrachloride (5 ml). The solution was kept for one hour and the red ppt. thus obtained was filtered washed and dried (0·98 g); crystallised from ethanol-benzene mixture. m.p. = 182°–183° C.

$C_{32}H_{22}N_4S_2O_2$ requires C = 67·02%. H = 3·94%, N = 5·78%.
Found C = 66·87%, H = 3·91%, N = 5·67%.

E. The debenzylation reactions of I *c* and II were carried out similarly as under D. In both the cases disulphide were isolated.

(I *c*) gave (III *c*) crystallised from ethanolic benzene as red crystals, m.p. = 199°–201° C.

$C_{26}H_{22}N_4S_2O_2$ requires C = 64·19%, H = 4·52%, N = 11·52%.
Found C = 64·93%, H = 4·38%, N = 11·57%.

(II) gave the disulphide (IV *a*), recrystallised from alcohol benzene mixture as deep red crystals with green lustre, m.p. = 243°–244° C.

$C_{40}H_{26}N_4S_2O_2$ requires C = 78·68%, H = 4·26%, N = 9·15%.
Found C = 78·92%, H = 4·13%, N = 8·96%.

F. 4-*Dimethylamino-azobenzene-2′-sulphenyl bromide :*

To a well stirred solution of I *d* (1·73 g) in CCl_4 (10 ml) kept at 40° C was added a solution of bromine (0·8 g) in carbon tetrachloride (5 ml). The mixture was kept at 40° C (30 min.). A violet red precipitate with a greenish lustre appeared, which was filtered off, washed and dried (0·72 g, 43% yield). It gave violet red crystals from alcohol-benzene mixture, m.p. 250°–252° C.

$C_{14}H_{14}N_3SBr$ requires C = 50·0%, H = 4·16%, N = 12·5%.
Found C = 49·76%, H = 4·45%, N = 12·11%.

G. Ultraviolet-visible spectra of the compound were taken in D.K-2 Beckmann Instrument.

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1. Specklin, R. and Meyback, J., *Bull. Soc. Chim.*, 1951, **18**, 627.
2. Burawoy, A., Turner, C., Hyslop, W. I. and Raymakers, P., *J. Chem. Soc.*, 1954, p. 82,

ADOPTIVE TRANSFER OF CELL-MEDIATED IMMUNITY TO *TRICHOPHYTON MENTAGROPHYTES* IN GUINEA PIGS

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ABSTRACT

Sensitized lymphocytes and their cell-free extract were shown to transfer cell-mediated immunity (CMI) to *Trichophyton mentagrophytes* in guinea pigs. Significant levels of delayed-type hypersensitive reactions (DTH) were also observed against antigens from heterologous dermatophytes, *viz*, *Trichophyton violaceum*, *Trichophyton rubrum* and *Microsporum canis* suggesting the cross reactivity.

INTRODUCTION

THE defense mechanism in response to infectious agents involves the differentiation and proliferation of B-cells (antibody-mediated immunity, AMI) or T-cells (cell-mediated immunity, CMI). In recent years, operation of these immune mechanisms in a number of infectious diseases, autoimmune processes, rejection of homografts and neoplastic tissue is well documented[1-3]. Resistance to some of the systemic mycotic infections like histoplasmosis and coccidioidomycosis has been attributed to the operation of both the mechanisms[4,5]. Although humoral antibodies and skin hypersensitive reactions have been reported in patients with ringworm infections, the role and nature of immune mechanism are not clearly understood[6]. Our studies on the immunity in experimental dermatomycoses in guinea pigs have demonstrated a low level of AMI and a high level of dermal sensitivity of delayed-type, implying the role of CMI during the recovery from infection (unpublished data).

With the exploration of cellular and biochemical aspects of immune mechanisms, the role of "transfer factor" in transferring the immunity has been extensively studied[7], and its clinical value in a number of diseases recognized[8,9]. In the present communication, the transfer of delayed hypersensitivity to *Trichophyton metagrophytes* in guinea pigs, by sensitized lymphocytes and a cell-free extract from them are reported.

MATERIALS AND METHODS

Immunization of guinea pigs.—The mycelium from a 7-day old culture of *Trichophyton mentagrophytes* was washed with acetone at — 15° C and dried under vacuum. The acetone-dry powder was extracted with 0·1 M phosphate bufferred saline (PBS) of pH 7·2. The extract was dialyzed, and its protein content was estimated by the method of Lowry *et al.*[10]. From the soluble extract, the proteins were separated by the method of Schneider[11], and lipopolysaccharide by the method of Westphal *et al.*[12]. The guinea pigs (350–400 g body wt.) were immunized by injecting the soluble extract (12 mg protein) from *T. mentagrophytes* with Freund's Complete Adjuvant in three doses at weekly intervals. Two weeks after the last dose of antigen, the animals were tested for delayed-type of hypersensitive reactions (DTH).

Transfer of CMI to non-sensitized guinea pigs.—The spleens from the animals showing a positive skin reaction (14–15 mm of induration and edema) were collected aseptically, minced and squashed in PBS. The suspension was filtered through a sterile stainless steel mesh and the cells were washed thrice with PBS and suspended in the same at a cell density of 90 × 10^6 lymphocytes per ml. A cell-free extract was prepared from lymphocytes (180 × 10^6 cells in 2 ml) by repeated freezing and thawing for 10 times. Either whole cells or cell-free extract (0·2 ml) were administered subcutaneously into the clipped and shaved flanks of non-sensitized guinea pigs. After an incubation period of 20 hr, the animals were challenged intradermally with 0·1 ml of soluble extract or protein fraction of lipopolysaccharide fraction from *T. mentagrophytes.* The cross reactivity with the related dermatophytes was examined by injecting the soluble extracts from *Trichophyton violaceum, Trichophyton rubrum* and *Microsporum canis.* The hypersensitive reactions developing 24 hr later were recorded.

RESULTS AND DISCUSSION

Guinea pigs injected with sensitized lymphocytes showed a positive DTH reaction on challenge with a soluble extract prepared from *T. mentagrophytes* (Table I). A similar reaction was also seen in animals which received the lymphocyte-extract.

The soluble extract contained both proteins as well as lipopolysaccharides. While the protein fraction elicited reactions comparable with soluble extract, no reactions were observed with lipopolysaccharide fraction.

TABLE I

Adoptive transfer of CMI to T. mentagrophytes *in guinea pigs*

Antigenic material	Skin reaction (in mm) in animals injected with	
	Lymphocytes	Lymphocyte-extract
T. mentagrophytes		
Soluble extract (20 μg)+	12	11
Protein fraction (20 μg)	11	10
Lipopolysaccharide fraction (20 μg)++	0	0
T. violaceum		
Soluble extract (20 μg)+	10	not tested
T. rubrum		
Soluble extract (20 μg)+	8	not tested
M. canis		
Soluble extract (20 μg)+	9	not tested

+ In terms of protein ++ In terms of hexoses

Soluble extracts prepared from other dermatophytes like *T. violaceum, T. rubrum* and *M. canis* also showed significant levels of DTH reactions suggesting cross reactivity among these fungi. But the degree of reactions do differ markedly as compared with the homologous system.

The present findings reveal the occurrence of a "transfer factor" in lymphocytes sensitized to *T. mentagrophytes* as observed in histoplasmin and coccidioidin sensitivity[13]. The chemical nature of this "transfer factor" and its *in vivo* efficacy in curing experimental dermatomycoses are being investigated.

ACKNOWLEDGEMENTS

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1. Craddock, C. G., Longmire, R. and McMillan, R., *N. Engl. J. Med.*, 1971, 285, 324.
2. —, — and —, *Ibid.*, 1971, 285, 378.
3. Witon, J. M. A., Ivanji, L. and Lehner, T., *Br. Med. J.*, 1972, 1, 723.
4. Catanazaro, A., Spitler, L. E. and Moser, K. M., *Cell Immunol.*, 1975, 15, 360.
5. Kirkpatrick, C. A., Chandler, J. W., Smith, J. K. and New Berry, W. M., "Cellular Immunologic Studies in Histoplasmosis," In : *Proceedings of the Second National Conference*, Charles C. Thomas, Springfield, Ill., 1971.
6. Grappel, S., Bishop, C. T. and Blank, F., *Bact. Rev.*, 1974, 38, 222.
7. Lawrence, H. S., "Transfer factor," In : *Advances in Immunology*, Eds., Dixon, F. J. and Kunkel, H. G., Academic Press, New York, 1969, p. 196.
8. —, *N. Engl. J. Med.*, 1970, 283, 411.
9. Hitzig, W. H. and Grob, P. J., "Therapeutic uses of Transfer factor," In : *Progress in Clinical Immunology*, Ed., R. S. Schwartz, Grune and Stratton, Inc., New York, 1974, 2, 69.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, 193, 265.
11. Schneider, W. C., *Ibid.*, 1945, 161, 293.
12. Westphal, O., Luderitz, O. and Bister, F., *Z. Naturf.*, 1952, 7 B, 148.
13. Stevens, D. A., Pappagianis, D., Manikovich, U. A. and Waddell, T. F., *Cell Immunol.*, 1974, 12, 37.

AWARD OF RESEARCH DEGREES

Tamil Nadu Agricultural University, Coimbatore, has awarded the Ph.D. degree in Agriculture to Shri S. Ramiah, Shri J. Chandramohan and Shri V. Jagannathan.

Osmania University, Hyderabad, has awarded the Ph.D. degree in Physics to Shri K. Ravindranath ; Ph.D. degree in Geophysics to Shri A. Srisailanath ; Ph.D. degree in Chemistry to Shri Iragavarapu Suryanarayana ; Ph.D. degree in Geology to Shri K. Madhavan Nair ; Ph.D. degree in Zoology to Shri J. Dasharatham Goud and Kumari Padma.

The M.S. University of Baroda, has awarded the Ph.D. degree in Physics to Kumari Mira Dhirajlal Trivedi and Shri Prabhakar Vaman Deshpande ; Ph.D. degree in Chemistry to Shri Joginder Singh and Kumari Prabha Shankar Gawande.

LETTERS TO THE EDITOR

UNIFIED TREATMENT OF EINSTEIN'S AND GÖDEL'S UNIVERSES

It is well known that the only stationary homogeneous world models are Gödel[1] and Einstein universes (Ozsvath[2]). Therefore it would be worthwhile to rederive these two solutions in a unified way. This is the object of the present note.

Both of these universes are filled with dust. Einstein's cosmos can be characterised by the postulate that expansion, rotation and shear of the dust distribution would vanish. The line element for this universe can be expressed in the form[2] :

$$ds^2 = (dx^0)^2 - (dx^1)^2 - (dx^2)^2 - 2\cos\beta x^1 (dx^2)(dx^3) - (dx^3)^2 \tag{1}$$

where β is a constant and it is related to the cosmological constant λ by $\beta^2 = 4\lambda$. Gödel[1] universe can be characterised by the postulate that expansion and shear of the dust distribution does vanish but the rotation of the dust distribution does not vanish. The line element for this universe can be expressed in the form[3] :

$$ds^2 = (dx^0)^2 + 2e^{ax^1}(dx^0)(dx^2) + \tfrac{1}{2}e^{2ax^1}(dx^2)^2 - (dx^1)^2 - (dx^3)^2 \tag{2}$$

where a is constant and it is related to cosmological constant λ by $a^2 = -2\lambda$.

For the unified treatment of these two world models we consider the metric in the form :

$$ds^2 = (dx^0 + \mathrm{X}dx^2)^2 - (dx^1)^2 - \mathrm{Y}^2(dx^2)^2 - (dx^3 + \mathrm{F}\,dx^2)^2 \tag{3}$$

where X, Y and F are functions of x^1 only. One can get both (1) and (2) out of (3) by a suitable choice of the functions X, Y and F.

We introduce the tetrads

$$\theta^0 = dx^0 + \mathrm{X}\,dx^2,\ \theta^1 = dx^1,\ \theta^2 = \mathrm{Y}\,dx^2,\ \theta^3 = dx^3 + \mathrm{F}\,dx^2 \tag{4}$$

so that (3) becomes

$$ds^2 = (\theta^0)^2 - (\theta^1)^2 - (\theta^2)^2 - (\theta^3)^2 = g_{(ab)}\theta^a\theta^b \tag{5}$$

We shall now try to solve Einstein's field equations corresponding to a dust distribution with non-zero cosmological constant λ. These field equations can be expressed in the tetrad form as

$$\mathrm{R}_{(ab)} - \tfrac{1}{2}\mathrm{R}\,g_{(ab)} + \lambda g_{(ab)} = -8\pi\rho\, v_{(a)} v_{(b)} \tag{6}$$

where R, ρ and $v_{(a)}$ are respectively the scalar curvature, dust density and the tetrad components of the flow vector. We use the comoving coordinates. Hence we have

$$v^{(0)} = 1,\ v^{(1)} = v^{(2)} = v^{(3)} = 0. \tag{7}$$

With the help of the results $v^i = e^i_{(a)} v^{(a)}$ and $dx^i = e^i_{(a)}\theta^a$ we can obtain the tensor components v^i of the flow vector. They are given by

$$v^0 = 1,\ v^1 = v^2 = v^3 = 0 \tag{8}$$

It is painless to verify that the rotation Ω of the congruence $v^i = (1, 0, 0, 0)$ is given by

$$\Omega^2 = 2\mathrm{X}'^2/\mathrm{Y}^2. \tag{9}$$

Here and in what follows, an overhead dash indicates differentiation with respect to x^1. Thus for Einstein's universe $\mathrm{X}' = 0$ and for Gödel's universe $\mathrm{X}' \neq 0$.

For the metric (5), the field-equations (6) alongwith (7) imply the following relations

$$\mathrm{F}' = \beta\mathrm{Y},\ \mathrm{X}' = \alpha\mathrm{Y},\ 4\lambda = \beta^2 - \alpha^2,\ \alpha\beta = 0$$
$$\mathrm{Y}'' + (\beta^2 - \tfrac{1}{2}\alpha^2)\mathrm{Y} = 0,\ 16\pi\rho = \alpha^2 + \beta^2 \tag{10}$$

where α and β are constants of integration.

The result $\alpha\beta = 0$ implies $\alpha = 0$ or $\beta = 0$. If α and β both vanish, we get a flat space-time. If $\alpha = 0$, then X becomes a constant. By a coordinate transformation in the metric (3) it can be seen that X = constant is equivalent to X = 0. Similarly $\beta = 0$ gives us F = 0.

In the case $\alpha = 0$, we have

$$\mathrm{X} = 0,\ \mathrm{Y} = \sin\beta x^1\ \mathrm{F} = \cos\beta x^1$$
$$\Omega = 0,\ 16\pi\rho = \beta^2 = 4\lambda \tag{11}$$

The solution described by (11) is the Einstein's universe given by the metric (1).

In the case $\beta = 0$, we have

$$\mathrm{F} = 0,\ \mathrm{X} = e^{\alpha x^1},\ \mathrm{Y} = (1/\sqrt{2})\,e^{\alpha x^1}, \tag{12}$$
$$\Omega = 4a^2,\ 16\pi\rho = 2a^2 = -4\lambda$$

where $\sqrt{2}a = \alpha$. The solution described by (12) is the Gödel's cosmos given by the metric (2).

The author is grateful to Professor P. C. Vaidya for invaluable critical comments and encouragement.

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1. Gödel, K., *Rev. Mod. Phys.*, 1949, **21**, 447.
2. Ozsvath, I., *J. Math. Phys.*, 1965, **6**, 590.
3. Adler, R., Bazin, M. and Schiffer, M., *Introduction to General Relativity*, McGraw-Hill, 1965, p. 367.

CHROMIC ACID OXIDATION OF NAPHTHALENE. EVIDENCE FOR THE INTERMEDIARY STEPS

In continuation of our study of the mechanism of chromic acid oxidation of aromatic hydrocarbons[1], we have investigated the kinetics of oxidation of naphthalene in 95% acetic acid. Using naphthalene in considerable excess over that of chromium trioxide, oxidation was substantially limited to the conversion to 1, 4–quinone, the subsequent oxidation to phthalic acid taking place to only a minor extent.

Kinetics of the disappearance of chromic acid was followed by iodimetry. The rate was found to be second order with respect to $HCrO_4^-$ (and not total Cr^{VI}). The order with respect to naphthalene was found to be kinetically a complicated one due to an initial complex formation between the reactants, prior to the rate limiting step. This complexation led to a decrease in the third order rate constant[2] k'_3 with increase in naphthalene concentration (*cf.* Michaelis-Menten behaviour of enzymes[3]).

The following scheme

$$C_{10}H_8 + Cr^{VI} \overset{K}{\rightleftharpoons} \text{COMPLEX}$$

$$\text{COMPLEX} + Cr^{VI} \xrightarrow{k'_3} C_{10}H_6O_2 + 2\,Cr^{III}$$

gives rise to a relation[4a]

$$1/k'_2 = \frac{1}{k'_3\,K\,(\text{NAPHTHALENE})} + \frac{1}{k'_3}$$

Figure 1 describes the reciprocal plots (obtained at various acid concentrations). From the slopes and intercepts, values of k'_3 and K were calculated (Table I).

TABLE I

Variation of k'_3 and K with acidity

(H^+) M	K M^{-1}	k'_3 $M^{-1}S^{-1}$
0·03	16·050	0·1562
0·10	16·440	0·6098
0·15	16·000	1·0000
0·20	15·930	1·2500

CrO_3 0·0060 M, HOAc 95%, μ 0·3 and T 30° C.

It was observed that rate constants k'_3 showed a first order dependence on (H^+) rather than on the acidity function H_0[4b] and K was acid independent. The overall rate law was given by

$$v = \frac{K\,k''_3\,(H^+)(\text{NAPHTHALENE})\,(HCrO_4^-)^2}{1 + K\,(\text{NAPHTHALENE})}$$

where $k''_3 = k'_3/(H^+)$. The dependence on (H^+) could arise from involvement of water in proton transfer in the rate limiting step.

The rates of oxidation of naphthalene–D_8 (Table II) did not permit a clear cut evaluation of the primary isotope effect. However, the ratio k'_{3H}/k'_{3D} is higher than the one expected on the basis of only a secondary deuterium effect.

TABLE II

Kinetic isotopic effect

	KM^{-1}	k'_3 $M^{-1}S^{-1}$	K_H/K_D	k'_{3H}/k'_{3D}
Naphthalene	16·44	0·83		
Naphthalene-D_8	8·44	0·37	1·74	2·25

CrO_3 0·003 M, HOAc 95%, H^+ = 0·1 M, μ = 0·3, T = 30° C.

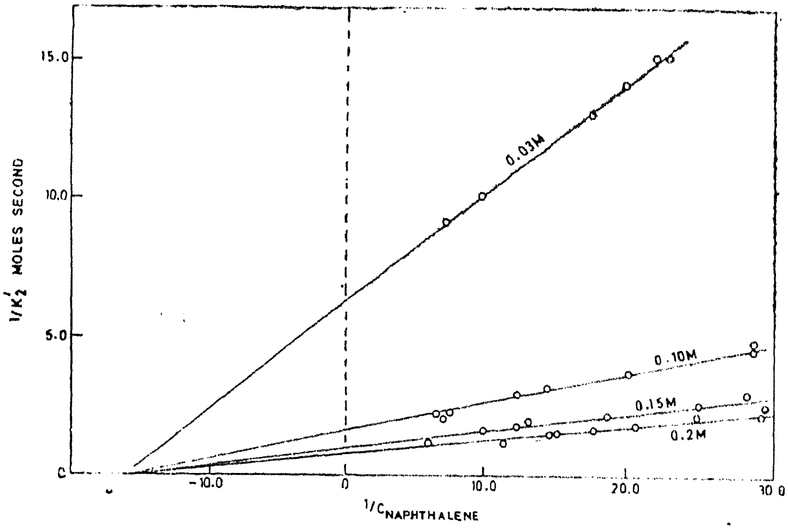
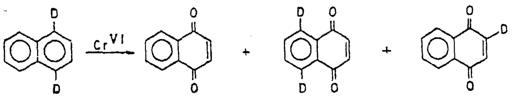


FIG. 1 RECIPROCAL PLOTS AT VARIOUS H^+ (T = 30 °C)

Naphthaquinone, obtained from naphthalene 1, 4–D_2 on oxidation, showed the intensity of the peaks in NMR for $\alpha : \beta$: quinonoid hydrogens = 2 : 4 : 3 (instead of 2:4:4 expected for equimolar mixture of I and II in Scheme I). Clearly, the hydrogen of the quinonoid moiety was partly substituted with deuterium. This view was confirmed by mass spectrum which showed the M^+ peaks corresponding to I, II and III (Scheme I) at 158, 159 and 160.

SCHEME I



I II III

Also, the fragmentation patterns fully supported this assignment (m/e, 130, 131 and 132 (M^+–CO), 102, 103, 104 (M –2CO), 104 and 106 (130 and 132–C_2H_2 and 131–C_2HD) and 76 & 78 (benzyne and benzyne–D_2). The formation of naphthaquinone–D provides the crucial evidence for the intermediacy of a naphthalene 1, 2–epoxide, or an 1, 2–epoxide like transition state. The migration of deuterium from 1 to 2 position is caused apparently by the 'NIH shift[5]', well recognised as a diagnostic feature of arene–oxide involvement. These findings place the chromic acid oxidation on a similar basis mechanistically to chromyl chloride oxidations[6].

Table III presents the kinetic behaviour in the presence of Mn^{II} (which is usually employed to trap Cr^{IV} and Cr^{V}). Instead of the expected decrease in rate, surprisingly the reaction was found to be catalysed by Mn^{II} ions. Further, the order with respect to Cr^{VI} changed from two to one on increasing Mn^{I} concentration. Difference spectra indicated as max 500 nm, which could be assigned to Mn^{III} species[7].

TABLE III

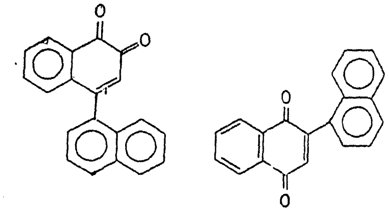
Effect of Mn^{II}

Mn^{II}	$k'_2 M^{-1} S^{-1}$	$k'_1 S^{-1}$	$k'_3 M^{-1} S^{-1}$
0·000	0·3745		9·3632
0·002	0·9257		23·1430
0·010	2·0200		50·5040
0·050		$8 \cdot 688 \times 10^{-3}$	72·4000
0·200		$8 \cdot 808 \times 10^{-3}$	73·4000

Naphthalene = 0·04 M, CrO_3 = 0·003 M, H^+ = 0·1 M, μ = 0·3, HOAc = 95%, and T = 30° C.

The reaction order with respect to Mn^{II} was found to be 0·2, indicative of a radical process. Product analysis showed that, along with naphthaquinone, two new quinones were produced. Their structures were found to be A and B (Scheme II) on the basis of IR, NMR, mass, UV spectra and confirmed also by synthesis. The formation of these quinones in presence of Mn^{II} could again mean radical processes, the intermediate radicals being trapped by the excess naphthalene present in the medium.

SCHEME II



QUINONE A QUINONE B

We are planning to discuss these results in a more detailed paper.

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April 18, 1977.

S. RAMESH.
M. VIVEKANANDA BHATT.

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1. Kutty, N. and Bhatt, M. V., *Tet. Lett.*, 1971, p. 2127.
2. Rate = k'_2 $(HCrO^-_4)^2$, k'_2 $M^{-1} S^{-1}$ = pseudo second order rate constant and k_3 $M^{-2} S^{-1}$ = k'_2/(Naphthalene).
3. Gray, C. J., *Enzyme Catalysed Reactions*, Van Nostrand Reinhold Company, London, 1971.
4a. Derived with an assumption that the concentration of the complex is small.
4b. Hammett, L. P., *Physical Organic Chemistry*, 2nd Ed., McGraw-Hill Book Company, New York, 1970.
5. Gurrof, G., Daly, J. W., Jarina, D. M., Renson, J., Witkop, B. and Udenfriend, S., *Science*, 1967, 158, 1524.
6. Sharplus, K. B. and Flood, T. C., *J. Amer. Chem. Soc.*, 1971, 93, 2316.
7. Fenton, A. J. and Furman, N. H., *Anal. Chem.*, 1960, 32, 748.

SYNTHESIS OF ISOXAZOLO (7, 6-*d*)-COUMARINS AND ISOXAZOLO (7, 8-*d*)-COUMARINS

ISOXAZOLO (7, 8–*d*) flavones are known to possess antibacterial activity[1]. Some furano (6, 7–*d*)–1, 2-benzisoxazoles and isoxazolo (6, 7–*d*)–1, 2-benzisoxazoles also exhibit antifungal activity[2]. In view of this, the synthesis of a few isoxazolo (7, 6–*d*) coumarins (I) and isoxazolo (7, 8–*d*) coumarins (II) was carried out for the first time.

The starting materials, 7–hydroxy–6–aryl–4–methylcoumarins were obtained by known method[3], while 7–hydroxy–8–acetylcoumarins were prepared by the Fries migration of 7–acetoxycoumarins[4]. The corresponding oximes on acetylation yielded the oxime acetates which on cyclization by the pyridine method reported by us earlier[5] gave the corresponding isoxazolo (7, 6–*d*) coumarins (I) and isoxazolo (7, 8–*d*)-coumarins (II). The physical data of these compounds is reported in Table I.

TABLE I

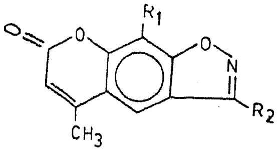
Physical data of hydroxy-acyl-coumarins and their corresponding oximes, oxime acetates and isoxazolo (7, 6-d)-coumarins (I) and isoxazolo (7, 8-d) coumarins (II)

7-Hydroxy-acyl-coumarins	M.P. °C	Yield %	Corresponding oximes M.P. °C	Corresponding oxime acetate M.P. °C	Corresponding isoxazolocoumarins *(I and II)	M.P. °C
6-Acetyl-4-methyl-	212[a]	22	256[b]	188[a]	I*a*	269[b]
6-Propionyl-4-methyl-	226[b]	25	230[b]	178[a]	I*b*	213[b]
6-Acetyl-4, 8-dimethyl-	193[b]	25	254[b]	212[b]	I*c*	256[b]
6-Propionyl-4, 8-dimethyl-	191[a]	23	245[b]	173[a]	I*d*	210[b]
8-Acetyl	167[a]	56	227[a]	161[a]	II*a*	228[c]
8-Acetyl-6-ethyl-4-methyl-	131[a]	83	172[a]	125[a]	II*b*	160[a]

* All compounds gave satisfactory elemental analyses.

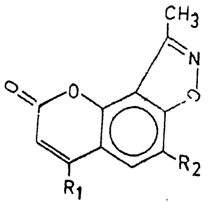
a = Crystallised from ethanol ; *b* = crystallised from acetic acid ; *c* = crystallised from 70% acetic acid.

IR spectra (nujol) of I and II showed bands around 1725, 1630, 1220 and 830 cm–1 characteristic of C=O coumarins, C=C, N–O–C and isoxazole ring stretch respectively.



I

	R_1	R_2
a	H	CH_3
b	CH_3	CH_3
c	H	C_2H_5
d	CH_3	C_2H_5



II

	R_1	R_2
a	H	H
b	CH_3	C_2H_5

Physiological activity.—Some of the compounds were tested against plant pathogenic fungi, *viz.*, *Helmenthosporium apatternae* and *Pestalotia* sp. at 50 ppm; but none of the compounds was found to possess any significant activity. The screening was done by dry weight method.

General procedure for the preparation of :

Oximes—The acylcoumarin (0·1 mole) was treated with hydroxylamine hydrochloride (0·12 mole) and sodium acetate (1 g) in an aqueous solution and refluxed for 40 min. The solid separated on cooling was filtered, washed with water and crystallized from a proper solvent. The yields of these oximes were between 60–72%.

Oxime acetates.—The oxime (0·05 mole) was heated with acetic anhydride (0·075 mole) on a water-bath till dissolved and kept aside for 30 min. It was then poured over crushed ice. The solid thus obtained was filtered, washed with water and crystallized from a proper solvent. The oxime acetates were obtained in 65–72% yield.

Cyclization of oxime acetates to corresponding isoxazolo (7, 6-d) coumarins (I) isoxazolo-(7, 8–d) coumarins (II)

The oxime acetate (1 g) was refluxed with dry pyridine (10 ml), for 3 hr. The reaction mass was then poured over crushed ice containing enough hydrochloric acid to neutralise pyridine present in it. The resulting solid was filtered, washed with water and crystallized from a proper solvent. The isoxazolocoumarins were formed in yields ranging from 70–85%.

The authors are thankful to Dr. D. S. Mukadam, Botany Department, Marathwada University, for co-operation extended in testing compounds for anti-fungal activity. They are also thankful to Dr. D. D. Goswami of this Department for scanning IR spectra.

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---

1. Kumari, S. S., Rao, K. S. R. M. and Rao, N. V. S., *Proc. Indian Acad. Sci.*, 1973, 77, 149.
2. Bhawal, B. M., *Ph.D. Thesis*, Marathwada University, 1977.
3. Desai, R. D. and Ekhlas, M., *Proc. Indian Acad. Sci.*, 1948, **8A**, 567.
4. Russel, A. and Frye, J. R., *Org. Syn*, Coll. Vol. 3, 1955, p. 282.
5. Thakar K. A., Goswami, D. D. and Bhawal, B. M., *Indian J. Chem.* (in press).

COMPLEXES OF PROTOCATECHUIC ACID WITH LANTHANIDES

COMPLEX formation by several dihydroxybenzoic acids with Al(III)[1,2] and lanthanide, complexes with the ortho hydroxybenzoic acids[3], orthohydroxy hapthoic acid[4] and Protocatechualdehyde[5] (3, 4 dihydroxy benzaldehyde) have been reported. In the present investigation, the preparation and characterisation of Y^{3+}, La^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+} and Gd^{3+} — Protocatechuates are reported.

The lanthanide complexes were prepared by the published procedure[5]. The analytical data of the complexes indicated that the ligands are attached to the lanthanide ion in the ratio of 3 : 1. Conductivity measurements indicated that all the complexes are non-electrolytes. Analytical data is represented in the Table.

The studies in the ultraviolet region were restricted to solutions in methanol and ethanol due to the

TABLE

COMPLEXES	ANALYSIS			U.V. ABSORPTION BANDS			INFRARED BANDS	
	Metal %	Carbon %	Hydrogen %	W.L. nm	Log ε_{max} Methanol	Log ε_{max} Ethanol	C–O Str.	-OH Str.
$Y(C_7H_5O_4)_3$	14.00	40·05	2·90	255	2·39	3·47	1660	2900
	(13·80)	(39·40)	(2·94)	290	3·36	3·51		3300
				355	2·38	1·91		
$La(C_7H_5O_4)_3$	16·82	38·00	1·93	255	3·72	3·80	1640	2900
	(16·85)	(38·46)	(1·81)	285	3·77	3·73		3400
				350	2·42	2·43		
$Pr(C_7H_5O_4)_3$	23·00	42·33	2·40	260	3·65	2·30	1660	2900
	(23·38)	(42·09)	(2·44)	290	3·65	3·65		3300
				355	2·39	2·30		
$Nd(C_7H_5O_4)_3$	22·15	37·00	2·30	255	3·77	4·08	1640	2900
	(21·83)	(38·15)	(2·27)	290	3·90	4·08		3350
				355	2·34	2·74		
$Sm(C_7H_5O_4)_3$	22·54	38·00	2·41	260	3·71	4·04	1650	2900
	(22·54)	(37·80)	(2·25)	290	3·90	4·01		3300
				355	2·39	2·30		
$Gd(C_7H_5O_4)_3$	20·58	37·00	2·33	250	3·84	2·53	1640	2900
	(20·30)	(37·41)	(2·22)	290	3·84	3·92		3300
				355	2·04	4·02		

Note : Numbers given in brackets are observed values.

sparingly soluble nature of the complexes. In this region the lanthanide ions do not show any characteristic absorptions when compared with that of the ligand. Hence only shifts at this wavelength are of significance[3]. Three band maxima are located with slight shift and intensification of absorption in the complexes. The details of the absorption maxima and log ϵ_{max} values are presented in the table.

Several investigations have been carried out in the visible region absorption spectra of the lanthanide complexes[3, 4, 6-9] to evaluate the relation between the structure and splitting pattern or intensification of absorptions. In the visible region, complex formation generally leads to alterations in the intensity or a shift in the position of absorption of the lanthanide ion or sometimes both. There has been no splitting of the bands in the Pr^{3+} and Nd^{3+} complexes as reported earlier[3]. A slight shift in the 796 nm bands of Nd^{3+} was observed, and the band at 745 nm remained unshifted, in contrast to the 2, 5 and 2, 6 dihydroxybenzoic acids[3]. The 402 nm band of Sm^{3+} was however located, due to low absorption of the ligand in this region.

Carboxylic acids[10] normally exhibit free OH stretch frequency in the region 3560–3500 cm^{-1} in the infrared region of the spectrum. In the ligand, there are two hands at 2900 and 3200 cm^{-1}, due to the OH stretching frequency. The lowering of the bands may be due to the internal hydrogen bonding in the ligand. In all the lanthanide complexes, the band at 2900 cm^{-1} was observed, and from this observation it can be concluded that one hydroxyl group remains intact. However, the increase in the OH stretching frequency may be due to the breaking of the internal hydrogen bonding of the ligand and its coordination with the metal ion, as the other band is located around 3300–33550 cm^{-1}.

In aryl acids the C=O vibration frequency is observed around 1700–1800 cm^{-1}. But in those acids which have internal hydrogen bonding the C=O frequency is lowered and located around 1670–1650 cm^{-1}. It was stated[10], that, in salicylic acid which is capable of internal hydrogen bonding the C=O frequency is observed around 1655 cm^{-1}. In the ligand, now investigated there is a band at 1670 cm^{-1} which is in conformity with the reported values[10], for acid showing internal hydrogen bonding. In all the lanthanide complexes reported, there is a lowering of C=O frequency, which is similar to the earlier report[3]. This lowering may be due to the possible coordination of the ligand with the metal ion. The I.R. spectral data are presented in the table.

A part of this work was carried out at Marathwada University, Aurangabad. The author is grateful to Dr. D. D. Khanolkar for his kind encouragement. He expresses his gratitude to Dr. V. Ramachandra Rao for his constant guidance during this work.

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July 21, 1977.

1. Reddi, M. L. N. and Seshaiah, U. V., *Indian Jour. Chem.*, 1964, 2, 34.
2. Soni, R. N. and Gupta, R. L. *Gzech. Chem. Commun.*, 1971, **36**, 2371.
3. Fazal Mehdi Ali, S. and Ramachandra Rao, V., *Jour. Inorg. Nucl. Chem.*, 1975, **37**, 1041.
4. Deshpande, Y. H. and Ramachandra Rao, V., *Ibid.* 1973, **35**, 3626.
5. Fazal Mehdi Ali, S., Gawande, M. P. and Ramachandra Rao, V., *Curr. Sci.*, 1973, **42**, 817.
6. Moeller, T., *J. Am. Chem. Soc.*, 1956, 72, 5447.
7. Vickery, R. C., *J. Chem. Soc.*, 1952, p. 423.
8. Moeller, T. and Ulrich, W. F., *J. Inorg. Nucl. Chem.*, 1956, 2, 164.
9. Purushottan, D., Ramachandra Rao, V. and Raghava Rao, Bh. S. V. *Anal. Chem. Acta.* 1965, **33**, 182.
10. Bellamy, L. J., *Infra Red Spectra of Complex Molecules*, Methuen, London, 1964, p. 162.

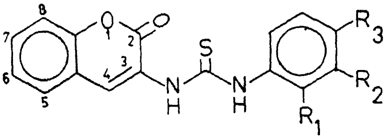
SYNTHESIS OF NEW COUMARYLTHIOUREAS WITH ANTITUBERCULOUS ACTIVITY

COUMARINS and substituted coumarins are known to be physiologically active and several of them possess antibacterial and antifungal properties[1]. Both 3– and 4–pyridylcoumarins are reported to be potential central nervous system depressants[2]. Also 3–aminocoumarins are found to have antibacterial properties[3]. Further, the usefulness of thioureas as antituberculous agents is well known[4]. It was, therefore, thought of interest to combine both the 3–aminocoumarin and the thiourea moieties into one structure and synthesise 3–coumarylthioureas having the general formula shown and test them for their antituberculous activity.

The synthesis of these compounds was carried out by condensation of 3–aminocoumarin[5] with different phenyl-isothiocyanates (prepared as described in literature) in boiling alcohol solution when the resulting thioureas were obtained as crystalline solids from alcohol or acetone in about 75–80% yields.

The I.R. spectrum of a typical coumarylthiourea showed the following bands: 3300 (–NH stretching), 1690 (–C=O of lactone), 1640, 1550, 1510 (aromatic), 1240 (C=S) cm^{-1}. The N.M.R. spectrum of the compound was also fully consistent with the structure.

Screening for antituberculous property of the compounds was done *in vitro*. Youman's liquid medium[6] was used for growing the organism, $H_{37}Rv$ strain of



No.	Colour	M.P.	Anti-T.B. activity $\mu g/ml$
1. $R_1 = R_2 = R_3 = H$	Colourless	197°	10
2. $R_1 = CH_3$, $R_2 = R_3 = H$	Pale yellow	168–9°	5
3. $R_1 = H$, $R_2 = CH_3$, $R_3 = H$	Cream	205°	5
4. $R_1 = R_2 = H$, $R_3 = CH_3$	Colourless	211°	10
5. $R_1 = H$, $R_2 = Cl$, $R_3 = H$	Pale yellow	217–8°	100
6. $R_1 = R_2 = H$, $R_3 = Cl$	Light brown	227–8°	1
7. $R_1 = R_2 = H$, $R_3 = OCH_3$	Yellow	172°	1
8. $R_1 = R_2 = H$, $R_3 = OC_2H_5$	Yellow	197°	1
9. $R_1 = R_2 = H$, $R_3 = OC_3H_7\,(n)$	Yellow	286–8°	100
10. $R_1 = R_2 = H$, $R_3 = OC_4H_9\,(n)$	Yellow	302–3°	100
11. $R_1 = R_2 = H$, $R_3 = OC_5H_{11}\,(n)$	Yellow	131–2°	10
12. $R_1 = R_2 = H$, $R_3 = OC_5H_{11}$ (iso)	Pale yellow	196°	10

Mycobacterium tuberculosis var. *hominis*. Eight days old culture of the test organism in Youman's liquid medium was used for inoculation. Thereafter, the incubation was carried out at 37° for 28 days. The results were noted by observation of pellicular growth at surface.

The minimum concentration of the compounds (mentioned in $\mu g/ml$) which completely inhibited the growth of the test organism are presented in last column of the table.

For comparison the activity under similar conditions for streptomycin is 1 $\mu g/ml$ and that of I.N.H. is 0·04 $\mu g/ml$.

All the compounds gave satisfactory elemental analysis. The screening tests were carried out at Haffkine Institute, Bombay.

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1. Okumara, K., Asin, K. and Okuda, T., *Yakugaku Zasshi*, 1961, **81**, 1482; Del Campo, A. and Fazzi, P. L., *Riv. ist. sieroterap. ital.*, 1958, 33, 389; Krishna Mohan Rao, K. S. R. and Subba Rao, N. V., *Curr. Sci.*, 1964, 33, 614.
2. Moppett, R. B., *J. Med. Chem.*, 1964, 7, 446; U.S., 1964, 315669.
3. Redighiero, G. and Antonello, C., *Bull. Chim. Farm.*, 1958, **97**, 592.
4. Burger, A., *Medicinal Chemistry*, Wiley-Interscience, New York, 1970, Vol. I, p. 431.
5. Linch, F. W., *J. Chem. Soc.*, 1912, **101**(II), 1765.
6. *American Review of Tuberculosis*, 1947, **55**, 529.

ANTHRAQUINONES IN *CASSIA SIAMEA*

THE Plant *Cassia siamea* (Fam. Ceasalpinaceae) is found in Indonesia, and also widely grown in the tropics of West and East Africa. Wehmer[1] has reported that bark and leaves of *C. siamea* contain anthraquinone like substances. In view of cathartic values of anthraquinones, an analytical work was carried out to characterise these anthraquinone compounds in leaf and bark of this plant.

Experimental.—Powdered leaf (1 g) and bark (1 g) were separately added to aqueous $FeCl_3$ (10 ml; 25%) + HCl (4 N each) and refluxed on a boiling water bath for 30 minutes. The mixtures were then extracted with chloroform (30 ml) and the extracts evaporated to dryness. The residues were taken up in methanol. Samples of leaf and bark extracts and of standard solutions of chrysophanol, physcion and rhein were applied to thin layer plates of silica gel (0·25 mm). Each chromatogram was first developed to 7·5 cm with benzene, ethyl formate, formic acid (75 : 24 : 1), and after drying, to 15 cm with light petroleum ether (40–60 °), ethyl acetate and glacial acetic acid (90 : 1·5 : 0·5). The yellow spots changed to orange-pink when sprayed with 5% KOH in methanol. In comparison with the reference solutions, spots observed in the leaf and bark extracts showed hRf identical to chrysophanol, 71·4; physcion, 66·1 and rhein, 33·7. Physcion was present only in the oxidative-hydrolysate of the bark and not the leaf. Benzene extracts of *C. siamea* bark had chrysophanol anthrone (hRf 80·2) and chrysophanol dianthrone (hRf 50·1) in addition to chrysophanol physcion and rhein when developed in the same solvent system on silica gel plates. About 20–25 mg of each of the compound detected on the chromatogram was isolated by preparative thin layer chromatography and its identity further confirmed by melting point, mixed melting point, U.V., visible and infrared spectra.

Quantitative estimation of free and combined anthraquinones was carried out by a colorimetric method[2] and also a densitometric method[3] described in earlier papers. The estimated content of free anthraquinones in *C. siamea* bark and leaves varied from 0·07 to 0·10 and of combined anthraquinones 0·60 to 1·0 giving a total of 0·67 to 1·10% w/w dry weight.

C. siamea is used as a purgative plant by the local tribes in Nigeria. The presence of various anthraquinones in this plant is obviously responsible for its purgative properties. The literature survey revealed that the present communication was the first systematic report on the anthraquinone constituents of this plant after Wehmar's early report of its presence in *C. siamea.*

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---

1. Wehmer, C., *Die Pflanzenstoffe*, 2 ed., Jena, Fischer, Suppl., 1935.
2. Rai, P. P., Turner, T. D. and Greensmith, S. L., *J. Pharm. Pharmacol.*, 1974, 26, 722.
3. —, — and Matlin, S. A., *J. Chromatog.*, 1975, 104, 196.
4. —, "Secondary products of therapeutic value in plant *Cassia siamea*", Presented at the 1st International Congress for Research on Medicinal Plants, Munich, W. Germany, September 1976.

KEN-SON LINEAMENT: A STUDY BASED ON LANDSAT-1 IMAGERY INTERPRETATION

GEOLOGICAL interpretation of a part of the Vindhyan Basin underlain by the Pre-Cambrian metamorphic basement in central India, carried out with the help of the multiband LANDSAT–1 imageries, revealed the presence of a number of linear features classified as lineaments and linears (Hoppins[1]). The linears and the lineaments were analysed in order to know the structural set up in this part of the Vindhyan Basin (Peshwa[2]).

The Multispectral Scanner aboard the LANDSAT–1 at a single instance, scans an area of 185 km × 185 km in such a way that the reflected solar energy in the range of 0·4–1·1 microns is split up into four wave bands as below:

0·5–0·6 (Band 4), 0·6–0·7 (Band 5), 0·7–0·8 (Band 6), and 0·8–1·1 (Band 7). The band No. 6 (partly) and band No. 7 (fully) covers the near infrared energy. This energy is recorded on the tape recorder in the satellite and later telemetered to the ground antenna where it is converted into four photo-analogues (imageries) which represent the four different wave bands.

During the present investigations, only the imageries in the band Nos. 5, 6 and 7 on 1:1,000,000 scale were utilised. Out of these, combination of band Nos. 5 and 7 gave the best results.

The analysis showed a number of linears, out of which the set of linears trending NNW-SSE were found to be very prominent. This trend is best represented by the 'Ken-Son lineament' in this note (Fig. 1), having a total length of nearly 200 km.

The Ken–Son lineament constitutes the linear courses of the Son river in the Satna and Shahdol districts of Madhya Pradesh and the Ken in the Banda district of Uttar Pradesh.

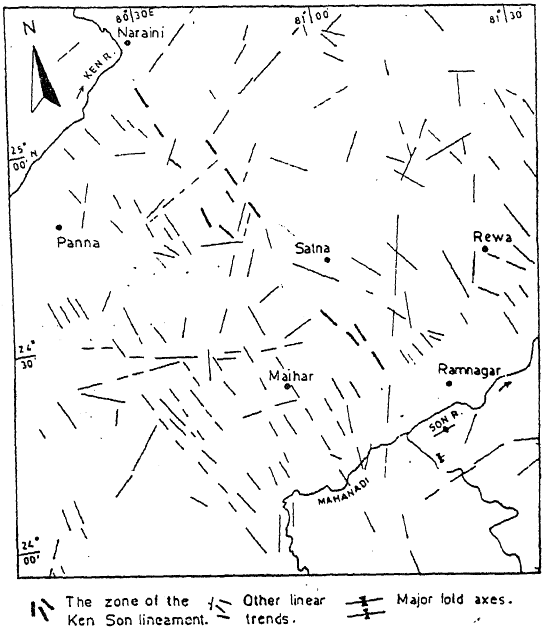
The river Son follows the northwesterly course between Ramgarh (23° 58′ and 81° 04′) and Markandeya (24° 05′ and 81° 30′), where it abruptly takes a turn towards ENE and continues to flow along the strike of the Vindhyans in this part. South of Ramgarh, between Duary (23° 58′, 81° 07′) and Imilia (23° 50′, 81° 15′) also, the river Son follows a linear course.

The river Ken, on the other hand, first follows the general trend of ENE–WSW parallel to the general strike of the Vindhyans and at Narayani (25° 05′ 81° 30′), it swings towards NNW and continues to flow in that direction.

The straight courses of these rivers lie in an alignment and together constitute the linears referred to as above. The area between the two linear courses shows the presence of a number of individual linears which form a part of the lineament.

On studying the exposures in the river Son south of Markandeya, it was discovered that the shales and siltstones belonging to the Semris, are traversed by a number of master joints which run for nearly 200 to 300 metres along the azimuth of the lineament. Their presence indicates that the lineament is a fracture zone which trends NNW–SSE.

Study of the area carried out with the help of the satellite imageries,, the aerial photographs on 1:60000 scale and the field work revealed the existence of ENE–WSW trending canoe folds, some of which run for nearly 30–50 km along their axes and have been shown in Fig. 1.



LANDSAT-1, I D 1057-04350, Band: 5·6·7. Scale: 1:1000,000.

FIG. I

The folds are believed to have formed at the outcome of SSE–NNW regional stress field. Paucity of such folds in the sediments, younger than the Semris indicate that the stresses became weaker after the deposition of the Semris. The 'Ken–Son lineament' described here represents the extension fracture system resulting from such a stress field.

The presence of this lineament with a number of small linears along its azimuth is discernible on the multiband satellite pictures much better than the aerial photographs. In the case of the band No. 7 of the satellite, the lineament looks dark, due to the moisture accumulation. The moisture absorbs the near infrared energy imparting a dark tone to the lineament. In band No. 5 of the imagery the vegitated areas along the fracture zone developed especially along the sandstones, look dark due to the absorption of the energy in that band by the chlorophyll in the vegitation.

Due to the vast areal coverage of the satellite picture, it was possible to detect a regional linear feature like the Ken–Son lineament which would otherwise have been missed on an aerial photograph which covers a limited area.

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---

1. Hoppins, R. A., *Bull. A.A.P.G.*, 1974, 58, 2260.
2. Peshwa, V. V., Unpublished Ph.D. Thesis, 19

CHRYSOTILE ASBESTOS OCCURRENCES IN THE ULTRAMAFIC ROCKS FROM SANGUEM DISTRICT, GOA, INDIA

THE note reports the occurrence of chrysotile asbestos in the ultramafic rocks from Sanguem (15° 00′ 00″ and 15° 20′ 16″ and 74° 04′ 00″ and 74° 15′ 00″) district of Goa. The mode of occurrence, the controls of localisation and the properties of the mineral are described in the following paragraphs.

The ultramafic rocks, which correspond to the metabasics of Pascoe[3], form the lowermost assemblage of the Dharwar granite-greenstone belt in this part of Indian Peninsula. They are found to occur about 2 km to SSE of Netravli village which is about 11 km to the SE of Sanvorde town in the Sanguem district of Goa.

The ultramafics show varying degrees of serpentinisation. The study of thin sections reveals that the serpentinised dunites at the base are the host rocks to the chrysotile asbestos mineralisation. From the field observations, it is seen that a strong mineralisation control is evident in the dunites affected by folding and shear deformation. Asbestos is found to occur as veins along the limbs of the minor folds. The hinge portions of such folds are not available for observations as they are more lateritised than the limbs. The asbestos fibres are found to have developed at right angles to the walls of the fractures. The width of veins varies from half centimeter to more than four centimeters. In other cases, asbestos fibres are found to have developed along strike slip faults, the length of fibres being parallel to the strike of the faults. In such cases, the fibres are of considerable length although they are found as a thin coating along the wall of the fracture.

The fibres are soft, whitish in colour and have a silky lustre. The specific gravity is 2·56. The properties of the asbestos correspond to those of chrysotile. When heated, the fibres suffered a loss in weight of 1, 10 and 13% at 500°, 700° and 850° C respectively. The mineral lost its lustre completely at 850° and became dull grey in colour. The fibres became brittle and could be easily crushed to powder with a slight pressure. From 850° C onwards upto 1100° C the weight remained constant. This indicates at 850° C the crystal structure of the mineral breaks down. The mineral has been found to be resistant to acid and alkali.

The examination of thin sections of serpentinites reveals that the asbestos is formed by the action of hydrothermal solutions on olivine present in the rock. According to Nagy and Faust[2], fibres appear to be best developed in serpentinites deficient in Fe and Al. The mineral veins may be considered to be formed by filling fractures through the agency of hydrothermal solutions aided by requisite structural deformation, as chrysotile asbestos is a stress-controlled mineral. Anhaeusser[1] is of the opinion that folding is the dominant regional controlling factor for asbestos development whereas faulting and fracturing provide the more localised control governing fibre growth and fibre density.

Finally, it is suggested that the present variety of asbestos is of long fibre type, which is easily separable. It can be used for the manufacture of yarn, asbestos cement, etc.

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1. Anhaeusser, C. R., "The nature of chrysotile asbestos occurrences in Southern Africa: A review," *Econ. Geol.*, 1976, **71**, 96.
2. Nagy, B. and Faust, F. G., "Serpentine: a natural mixture of chrysotile and antigorite," *Am. Mineralogist*, 1965, **41**, 817.
3. Pascoe, E. H., *A Manual of Geology of India and Burma*, Government of India Press, New Delhi, 1965, **1**, 485.

A PRELIMINARY REPORT ON THE FAUNA IN RADIOLARITES OF OPHIOLITE-MELANGE ZONE AROUND MULBEKH, LADAKH

THE note gives a first report of the planktonic foraminiferal and radiolarian assemblage from cherts and jasperites associated with the ophiolite-melange suite from Mulbekh (34° 07′ 30″ N.: 76° 22′ 30″ E.) in Ladakh district of Kashmir. The ophiolite-melange belongs to the Indus ophiolite belt sandwiched between the Kashmir-Rupshu basin to the south and Ladakh granitic mass with its sedimentary cover to the north. The geology of the area is discussed by Shah *et al.*[1].

The ophiolite-melange consists of tectonically emplaced serpentinite bodies and associated sedimentary material. The latter consists of chert, cherty shale, jasperite and occasional limestone. The fauna was recovered from a number of beds of chert and jasperite around Mulbekh and north of Shergol (34° 24′ 00″ N.: 76° 19′ 20″ E.). It could be studied only in thin sections since the rock did not respond to maceration. As such the specific identification of foraminifers could not be made and the generic identification of radiolarians is doubtful. The foraminifers identified include *Globotruncana* sp., *Heterohelix* sp. and *Rugoglobigerina* sp. while the radiolarians include *Liosphaera* sp., *Rhodosphaera* sp., *Cenosphaera* sp., *Dictyomitra* sp., *Sethocyrtis* sp. and *Flustrella* (*Flustrella*) sp. Tewari *et al.*[2] have reported *Globotruncana fornicata* and other planktonic and benthonic foraminifera from Gya in Ladakh from the rocks of the 'Indus Flysch'; the present report, however, is the first one from the 'melange' zone. *Globotruncana* and *Heterohelix* indicate a Middle to Upper Cretaceous age. These genera are also known from Sangcha Malla Formation[3] of Kumaun where their specific determination has been made.

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1. Shah, S. K., Sharma, M. L., Gergan, J. T. and Tara, C. S., *Him. Geol.*, 1976, **6**, 534.
2. Tewari, B. S., Pande, I. C. and Kumar, R., *Pub. Cent. Adv. Stud. Geol.*, Chandigarh, 1970, 7, 197.
3. Shah, S. K. and Sinha, A. K., *Him. Geol.* 1974, 4, 1.
4. Mamgain, V. D. and Sastry, M. V. A., *Pal. Ind*, *Geol. Surv.. Ind. N.S.*, 1975, **43**, 1.

THE FIRST EVIDENCE OF A HAFTED BLADE FOUND AT DAIMABAD, DISTRICT AHMEDNAGAR, MAHARASHTRA

THE excavations at Daimabad on the left bank of the river Pravara, a tributary of the river Godavari, being carried out by the author from 1974–75 onwards has brought to light a succession of five phases of chalcolithic culture, each one distinguished from the other by the occurrence of a distinct class of painted pottery tradition of its own (from the bottom upwards).

Phase I	Savalda Culture
Phase II (Later)	Harappa Culture
Phase III	Buff and Cream Ware Culture
Phase IV	Malwa Culture
Phase V	Jorwe Culture

The collection of parallel sided blades from these phases consists of both broken and unbroken specimens. But the unique specimen among these is a piece of blade which was found lying in the cavity of a flat bone piece. It was obtained from the topmost levels of the Jorwe Culture. The specimen represents an undoubted evidence of a blade hafted in a bone-handle (Fig. 1).

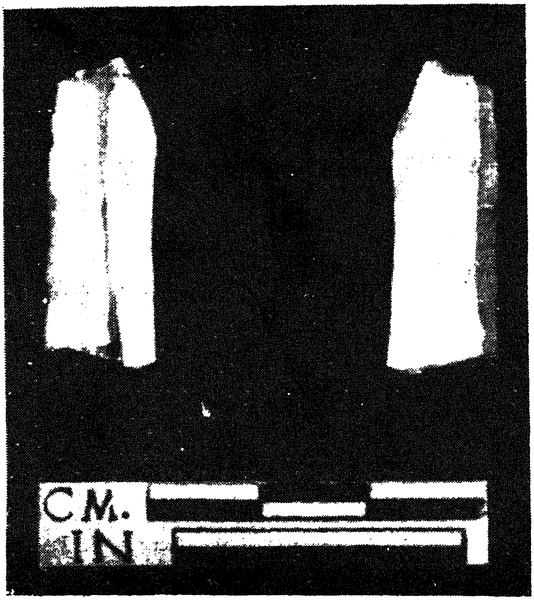

FIG. 1

The blade piece hafted in the spongy cavity of the flat bone is 24 mm long and 6 mm broad. Both its distal and bulber ends have been snapped. It is parallel sided and triangular in section with medial ridge on the upper side. Its back is not retouched. The edge which remains projected about 2 mm outside the bone along its length (hereafter called the working edge) is marked by a couple of tiny indentations and except these there are no signs of retouch or secondary work no the working edge.

The bone piece in which the above described blade piece lies is 25 mm long and 7 mm broad. Its underside is flat and the upper oblique. The high medial ridge of the blade touches the edge of the oblique upper side and thus the blade is prevented from getting further inside upto the other end of the cavity so that the other side of the bone-handle is not cut by the opposite edge of the blade from inside the cavity. The shape of the bone suggests that it is a piece of a rib-bone.

The blade remained in its original place because of the compact earth which had covered the specimen. There is no trace of original sticking matter.

The microlithic industries of the Chalcolithic period are blade industries. No definite evidence showing as to how the blades were used had hitherto been found in India. Yet on the basis of the evidence from Europe, Africa and West Asia it was generally believed that in this country also the blades and blade tools were used by hafting in a wooden or bone-handle. The parallel-sided fragment of a blade found inside the piece of a bone-handle in the upper levels of the Jorwe Culture at Daimabad, however, provides for the first time in India a direct evidence of hafting of blade in bone-handle.

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ON THE BOTANICAL FINDINGS FROM EXCAVATIONS AT DAIMABAD, A CHALCOLITHIC SITE IN WESTERN MAHARASHTRA, INDIA

CARBONISED grains of sixteen wild and cultivated species of plants, some of them being the oldest records in the Indian subcontinent as well as in Africa, have been recently collected by the author from Daimabad (19° 31′: 74° 42′), a Chalcolithic site in Ahmednagar District of Western Maharashtra. The site, tentatively dated as circa 2,200 B.C.–1.000 B.C. is being excavated since 1974 by Shri S. A. Sali of Archaeological Survey of India. The grains were collected by using flotation technique. Identification of the charred grains was carried out by noting the external morphological features under low power (25 ×) binocular microscope. A concordance chart of the various grain findings and the cultural sequence is given below (Table I).

TABLE I

Summary of stratigraphic grain findings from Daimabad, Maharashtra

Sl. No.	Common Name	Botanical Name	Cultural Phases				
			Savalda Circa 2200 B.C.	Late Harappan	Buff and Cream Ware	Malwa	Jorwe Circa 1000 B.C.
1.	Wheat	*Triticum* sp.				+	+
2.	Barley	*Hordeum vulgare* Linn.	+	+	+	+	+
3.	Rice	*Oryza sativa* Linn.					+
4.	Ragi (Finger millet)	*Eleusine coracana* Linn.					+
5.	Kodon millet	*Paspalum scrobiculatum* Linn.					+
6.	Sorghum (Jowar)	*Sorghum* sp.					+
7.	Common pea	*Pisum arvense* Linn.				+	+
8.	Grass pea	*Lathyrus sativus* Linn.			+	+	+
9.	Horse gram	*Dolichos biflorus* Linn.			+	+	+
10.	Hyacinth bean	*Dolichos lablab* Linn.			+	+	+
11.	Chick pea (Gram)	*Cicer arietinum* Linn.					+
12.	Lentil	*Lens esculenta* Moench.	+		+		+
13.	Black gram/Green gram (Urd/Moong)	*Phaseolus mungo* Linn./ *Phaseolus aureus* Roxb.			+		+
14.	Linseed	*Linum usitatissimum* Linn.					+
15.	Safflower	*Carthamus tinctorius* Linn.					+
16.	Indian jujube (Ber)	*Zizyphus jujuba* Lamk.	+	+		+	+

+ Indicates presence.

Out of the collection, the most important finding is that of Sorghum (*Sorghum* sp.) which forms the earliest record of its charred grains in India. The evidence supports Dogett's[1] hypothesis that Sorghum could have been introduced into India from Africa by the end of 2nd millenium B.C. Evidences gathered from comparative morphology, cytogenetics, distribution, history, linguistics, etc., suggest that Finger millet (*Eleusine coracana* Linn.) commonly called in India as Ragi, originated in Africa from where it was introduced into India[2]. The present record of *Eleusine coracana* Linn. is, therefore, important from the point of view of its time and place of domestication in India and Africa. The grains of Gram (*Cicer arietinum* Linn.) and Safflower (*Carthamus tinctorius* Linn.) also predates all other archaeobotanical findings of the same species in India. The present findings are all the more important because they illustrate sequential history of earliest agriculture in western Maharashtra. A detailed study of the findings is under progress.

The author is grateful to Shri M. N. Deshpande, Director General of Archaeological Survey of India and Shri S. A. Sali of South-Western Circle of Archaeological Survey of India for kindly entrusting the material to him for this investigation.

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1. Dogett, H., In Sir Joseph Hutchinson (Ed.), *Crop Plant Evolution*, Cambridge University Press, London, 1965.
2. Khidir, W. Hilu and De Wet, J. M. J. *Economic Botany*, 1976, **30**(3), 199.

EMBRYOLOGY OF *MELIANTHUS MAJOR* L.

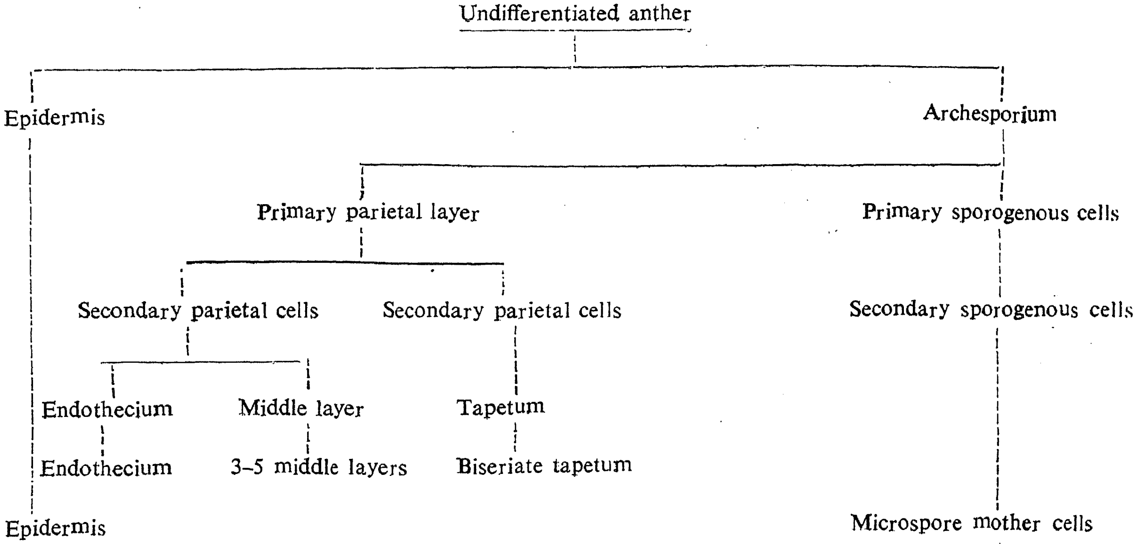
I. Microsporangium and Pollen

Melianthus major L. was placed by Bentham and Hooker[1] in the family Sapindaceae, but was separated on morphological grounds, by Gurke[4] and placed in a separate family Melianthaceae. It was later supported by most of the taxonomists. Recently Steyn[6] on the basis of embryogeny and Corner[2] on seed structure have also supported it, showing its affinities with Rosale.

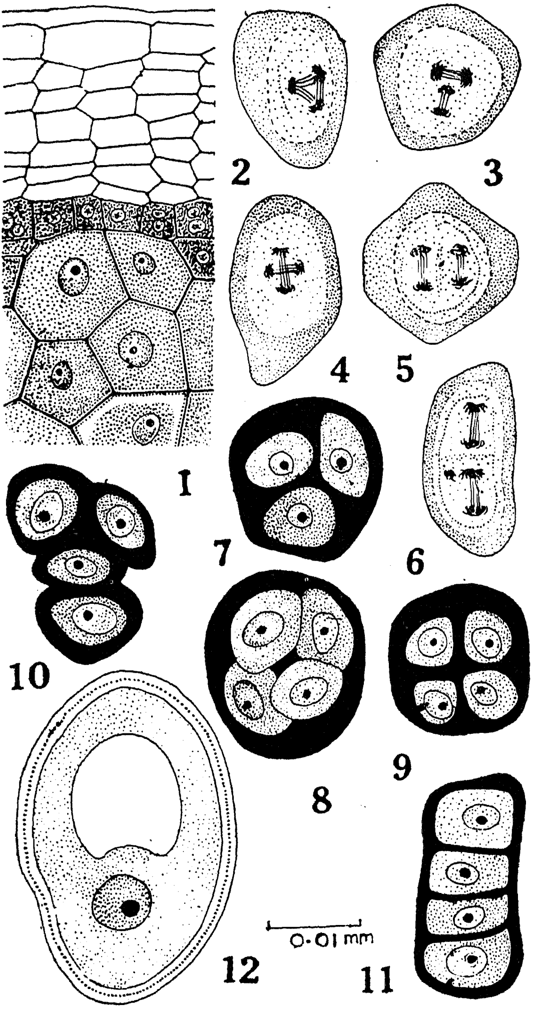
Flowers of *Melianthus* are tetramerous, bisexual, irregular and polypetalous, produced in dence racemes. Stamens, with long filaments, are four in two groups of unequal lengths. Ovary is tetralocular with 5 or 6 anatropous ovules on axile placentum in each locule.

A young tetrasporangiate anther differentiates into epidermis and central homogeneous mass, which cuts off parietal layer and inner multiseriate archesporium. The periclinal divisions in the parietal layer result in the differentiation of anther wall which consists of 6–8 layers of cells (Fig. 1). The epidermal cells are tangentially stretched with thin cuticle. As the development proceeds they become more stretched and narrow. The endothecium remains unchanged till the formation of pollen grains. Later the cells of endothecium elongate radially and produce the characteristic fibrous thickenings; rarely the thickening develop prior to the dehiscence. The number of middle layers varies from three to five which are usually ephemeral. Occasionally one or two of the middle layers persist till the anther derisces. The innermost wall layer is the glandular tapetum. It is uni-biseriate laterally and multiseriate towards anterior and posterior ends of the anther. Its cells are uninucleate to begin with but become bi-tetranucleate by mitotic or amitotic divisions during meiosis I. At the formation of microspore tetrads, minute globules appear on the inner tangential wall of the tapetum. The globules however are not observed at later stages of development. The cells later on become vacuolated, stretched and persist even after the formation of pollen grains.

The Following chart summarises the development of anther wall. It is of Dicotyledonous type (Davis[3]).



The protoplasm of microspore mother cells rounds off before they undergo meiosis. Meiotic divisions are normal; however the cytokinesis is simultaneous by furrowing, resulting in tetrahedral decussate and isobilateral types of tetrads and successive by cell plate formation producing linear, T–shaped and isobilateral types of tetrads (Figs. 2–11). A callose is differentiated at the reduction division and becomes more prominent at tetrad stage. Young microspores get separated by irregular disintegration of callose. They are rounded, with prominent nuclei and dense cytoplasm. The microspores enlarge and attain their characteristic elliptical shape which is triangular in polar view. The spore wall gets differentiated into exine and intine. Prior to the division the microspore nucleus migrates to one side and a big vacuole appears on the other side (Fig. 12). The nucleus divides resulting in a vegetative, pole ward and generative nuclei. Pollen grains are usually shed at this stage but three-nucleate pollen grains at the time of shedding are not uncommon. Pollen grain is monad, isopolar, radiosymmetric, perprolate ($49 \times 26\ \mu$) Amb peritreme-pleurotreme, apocolpium, diameter 6–7 μ, Tricolporate, colpi ± 42 μ long. narrow slit-like, ends pointed, Ora lalongate (± 4 × 14 μ), polar margins of the ora thickened.

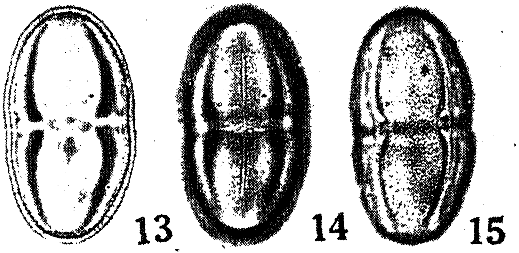


FIGS. 1–12. Microsporogenesis in *Melianthus major.* Fig. 1. Portion of anther wall at microspore mother cell stage, Figs. 2–6. Dividing mother cells. Figs. 7–11. Different types of tetrades. Fig. 12. Male gametophyte.

Exine 3 μ thick, sexine ± 1·5 μ thick, pertectate. Tectum supported by densely placed bacules. Nexine as thick as sexine (Figs. 13–15).

The microsporangia of each anther lobe are unequal in size, thus there is no coalescence of the adjacent locules and each microsporangium dehisces independently. At the region of dehiscence the cells of endothecium are small, they fail to develop fibrous thickenings and become cuticularised.

Morphologically quite distinct from Sapindaceous taxon, the genus *Melianthus* also differs in the development of microsporangia, microsporogenesis and male gametophyte from the worked out members of Sapindaceae in the presence of features such as persisting epidermis, 3–5 middle layers, biseriate tapetum, linear and 'T'-shaped tetrads, cytokinesis by furrowing as well as cell plate formation, 3-nucleate pollen grains at shedding, no coalescence of adjacent sporangia, and independent dehiscence of each lobe.



FIGS. 13–15. Pollen grains of *Melianthus major*

The author is grateful to Dr (Mrs.) Nirmal Gulhati under whose guidance this work is carried on; to Prof. Jafar Nizam, for providing laboratory facilities and encouragement. to Dr. M. R. Saxena for helping in description of pollen grains; to U.G.C. for granting Teachers Fellowship Award and to Vanita Mahavidyalaya College authorities for sanctioning it.

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August 22, 1977.

1. Bentham, G. and Hooker, J. D., *Genera Plantarum,* London, 1862.
2. Corner, E. J. H., *Seeds of Dicotyledons,* Cambridge, 1976.
3. Davis, G. L., *Systematic Embryology of the Angiosperms,* U.S.A., 1966.
4. Gurke, M., "Melianthaceae", In Engler and Prantl, *Die Naturlichen Pflanzon Families,* 1895, 3(5).
5. Khushlani, I. K., *Phyton,* 1963, 10, 143.
6. Steyn, E., *Jl. S. Afr. Bot.,* 1975, 41, 199.

VIBRIO PARAHAEMOLYTICUS IN THE MARINE ENVIRONMENT AT PORTO NOVO

INFORMATION on the occurrence of *Vibrio parahaemolyticus* from India is scanty. Chatterjee *et at.*[1] were the first to isolate *V. parahaemolyticus* and other associated non-cholera vibrios from the faeces of patients with diarrhoea in Calcutta. Recently, Bose and Chandrasekar[2] reported the occurrence of *V. parahaemolyticus* in the slime of marine prawns from the coastal waters of Nagapattinam. "A multicentric study on *V. parahaemolyticus*" is now underway at the Hoffkine Bio-Pharmaceutical Corporation Ltd., Bombay and at Cholera Research Centre, Calcutta, to study this pathogen in India.

The present study indicates the occurrence of *V. parahaemolyticus* and allied vibrios in shell fish. plankton and sediments of Porto Novo (11° 29′ N—79° 49′ E) along the east coast of South India.

Three species of shell fish, *viz.*, the oysters *Crassostrea madrasensis*, the Cockle, *Anadara rhombia* and the crab, *Portunus sanguinolentus* were collected from the fish landing centre and were immediately washed well with 50% sterile sea water. Swabs of the shell surface, mantle and faeces were then taken from *C. madrasensis* and *A. rhombia.* In addition to the swabs of the shell surface, the faeces and haemolymph of *P. sanguinolentus* were also screened. Plankton samples were harvested using a bolting silk No. 10 horizontal tow plankton net (0·158 mm mesh).

The sediment samples were collected using a Petersen grab from 3 different biotopes, namely mangrove, backwater and estuary.

A preliminary survey to study the distribution of *V. parahaemolyticus* in the sediments of the Vellar estuary (fish landing site) was also attempted. These sediment samples were collected on 5 occasions (From December, 1975 to February, 1976), serial dilutions were prepared and then pour plated with TCBS medium.

Samples derived from the various sources described above, were streaked on to TCBS medium (the composition of which is given in Table I) and incubated at 37°C for 24 hours. Following the suggestions of Kaneko and Colwell[3] colonies appearing on TCBS agar were regarded as presumptive vibrios (PV) or Vibrio-like organisms (VLO). "Typical" smooth, turbid, bluish green colonies on TCBS agar were regarded as presumptive *V. parahaemolyticus* (PVP) or *V. parahaemolyticus*-like organisms (VPLO). The ensuing isolates were restreaked repeatedly to obtain pure cultures.

The PV or VLO were identified according to standard methodology[4]. The identification protocol for PVP or VPLO was based on the 18 biochemical tests recommended by Kaneko and Colwell[5] for the rapid and reliable identification of *V. parahaemolyticus.* In order to confirm the identification, parallel tests were conducted alongside using a reference Japanese strain obtained from Zen Yoji through CFTRI, Mysore. The results obtained from the biochemical screening for *V. parahaemolyticus* are shown in Table II.

TABLE I

Composition of thiosulfate citrate bile salt sucrose agar (TCBS)

Ingredients	Quantity (g/1000 ml)
Yeast extract	5
Peptone	10
Sodium citrate	10
Sodium thiosulfate	10
Oxgall	5
Sodium cholate	3
Sucrose	20
NaCl	10
Ferric citrate	1
Bromothymol blue	0·04
Thymol blue	0·04
Agar	15
pH	8·6

TABLE II

Results of the biochemical tests employed for identification of V. parahaemolyticus *(as recommended by Kaneko and Colwell*[5]*) in comparison to the Japanese strain (Serotype unknown)*

Test	Reaction	
	Porto Novo Strains	Japanese Strain
Gram Stains	— (21)	—
Motility	+ (21)	+
Growth in NaCl :		
0%	— (21)	—
3%	+ (21)	+
7%	+ (21)	+
10%	— (21)	—
Oxidase Test	+ (21)	+
Fermentation of :		
Glucose (Acid)	+ (21)	+
Lactose (Acid)	— (21)	—
Sucrose (Acid)	— (21)	—
Mannitol (Acid)	+ (17)	+
H_2S Production	— (21)	—
Voges-pnoskauer Test	— (18)	—
Indole production	+ (21)	+
NO_3 Reduction	+ (21)	+
Catalase Test	+ (21)	+
Methyl Red Test	+ (18)	+
Bioluminiscence	— (21)	—

N.B. : Figures in parenthesis denote the number of positive *V. parahaemolyticus* isolates out of a total of 21 PVP or VPLO isolates tested.

The occurrence and distribution of *V. parahaemolyticus* and allied vibrios *V. alginolyticus*, *V. fischeri*, *V. costicola* and *V. anguillarum*) from the various sources are shown in Table III

VLO—$2 \cdot 3 \times 10^2$ to 240×10^2 per gram dry weight of sediment.

VPLO—$1 \cdot 3 \times 10^2$ to 238×10^2 per gram dry weight of sediment.

TABLE III

Occurrence of V. parahaemolyticus *and related vibrios in shell fish, plankton and sediments*

Sample	Source	No. of samples	No. of isolates (VPLO)	*Vibrio* Spp.				
				V. parahaemolyticus	*V. alginolyticus*	*V. costicola*	*V. fisheri*	*V. anguillarum*
SHELL FISH								
A. rhombia	Shell surface	7	5	0	0	0	5	0
	Mantle	7	1	1	0	0	0	0
	Faecal matter	7	0	0	0	0	0	0
C. madrasensis	Shell surface	12	9	7	0	0	1	1
	Mantle	12	6	6	0	0	0	0
	Faecal matter	12	0	0	0	0	0	0
P. sanguinolentus	Shell surface	5	3	0	0	0	1	2
	Faecal matter	5	4	3	0	0	1	0
	Haemolymph	5	5	4	0	0	1	0
	Egg mass	1	2	0	0	0	2	0
Sediments	Mangrove		20	6	5	3	5	1
	Backwater		25	4	10	8	1	2
	Estuary		40	25	4	4	3	4
Plankton		1	5	3	0	0	0	0

VLO and VPLO counts in sediment samples collected from the fish landing centre during three-month period are presented in Table IV. VLO and VPLO counts ranged as follows.

TABLE IV

Counts of VLO and VPLO in sediment samples from Porto Novo Fish Landing Centre

Date of Collection	VLO- counts* $\times 10^2$	VPLO counts* $\times 10^2$
12-12-1975	81·80	4·88
31-12-1975	63·15	18·12
14-01-1976	239·86	238·48
10-02-1976	2·28	1·28
29-02-1976	16·96	6·56

*Counts per gram dry weight of Sediment.

Maximum counts of VLO and VPLO were encountered during the middle of January (Postmonsoon period).

The *V. parahaemolyticus* strains isolated at Porto Novo closely resemble those isolated by Bose and Chandrasekar[3] at Nagapattinam, in being gram-negative, glucose (acid)-positive, Lactose (acid)-negative, mannitol (acid)-positive, oxidase-positive, nitrate reduction-positive and in exhibiting growth in 3% and 7% sodium chloride but not in 0% and 10% sodium chloride. The Porto Novo strains differed in being sucrose-negative and methyl red-positive.

The detection of this pathogen in shell fish, plankton and sediments of Porto Novo (which is an important landing centre for fish and prawns) calls for more detailed investigations on the ecology and seasonal distribution of *V. parahaemolyticus* in this region. Its potential hazard if any, in public health will have to be ascertained.

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1. Chatterjee, B. D., Neogy, K. and Gorbach, S., *Indian J. Med. Res.*, 1970, **58**, 234.
2. Bose, S. V. C. and Chandrasekar, F., *Fish Technol.*, 1976, 1, 36.
3. Kaneko, T. and Colwell, R. R., *Appl. Microbiol.*, 1974, **28**, 1009.
4. Shewan, J. M. and Veren, M. In the 8th edition of *Bergey's Manual of Determinative Bacteriology*, Buchanan, R. E. and Gibbons, N. E., Baltimore, 1974, p. 340.
5. Kaneko, T. and Colwell, R. R., *J. Bacteriol.* 1973, **113**, 24.

POLLEN MORPHOLOGY OF AN *ERYTHRINA* HYBRID AND THEIR PARENTS

THE trophical genus *Erythrina* is characterised by dazzling red inflorescences, for which the plant is highly valued as an ornamental. While most species are arbrorscent, *E. resupinata*, a species from Terai forests, is of special importance for being an underground herbaceous perennial, with inflorescences arising directly from the root-stock. Several species of the genus have been brought into cultivation at the National Botanic Gardens, and an attempt has been made to cross the dwarf *E. resupinata* Roxb. (♀) with the tall *E. variegata* L. var. *orientalis* (L.) Merrill *forma parcelli* (Hort. ex Bull) Maheshwari (♂). The hybrid is shrubby in habit, but the characters of the flower show general dominance of the female parent[1].

Considering all the materials together, the pollen grains are three zonoporate and are with a reticulate exine surface[2]. The detailed data of pollen morphology (Fig. 1, A–C) of the two species and their hybrids are as follows:

E. resupinata.—3–(4)–zonoporate; pore diameter about 7 μ. Grain sizes: average 21 × 30 μ (Size classes E: 24 μ: 1%; 26 μ: 7%; 28 μ: 48%; 30 μ: 23%; 32 μ: 20%. 34 μ: 1%). Shape—oblate. Exine surface faintly reticulate; lumina smaller.

E. parcellii.—3–(4)–zonoporate; pore diameter about 6·6 μ. Grain sizs: average 32 × 39 μ. (Size classes E: 32 μ: 2%; 36 μ: 22%; 39 μ: 12%; 40 μ: 52%; 42 μ: 4%; 44 μ: 8%). Shape—oblate. Exine surface faintly reticulate; lumina larger, and with irregular muri.

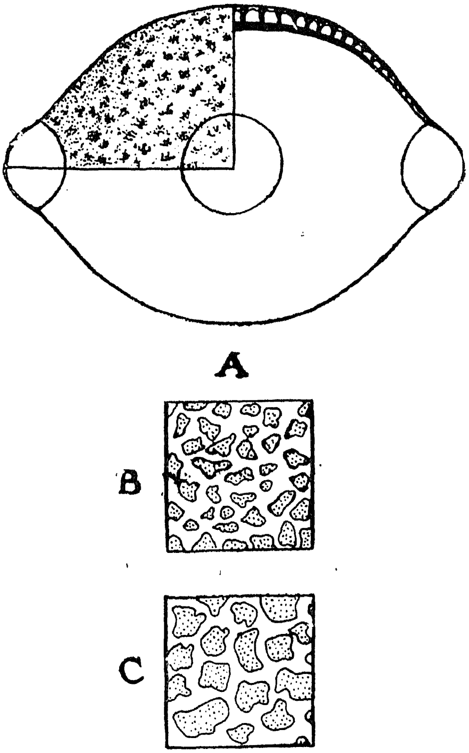

FIG. 1. A–C. Palynogram of *Erythrina*. A & B. *E. resupinata* (A. Equatorial view showing pore, general surface and strata; B. Reticulate ornamentation; note: brochi are small); C. *E. Variegata* (Reticulation; note: brochi large). Magnification: A × 1,500; B & C, × 3,000.

Hybrid.—Size classes: 28 μ: 14%; 30 μ: 25%; 32 μ: 52%; 34 μ: 19%; 39 μ: 17%.

The above data indicate that the dominant size class is 28 μ (range 24–34 μ), and the exine surface is provided with smaller brochi in *E. resupinata*, as against the higher average dominant size class of 40 μ (range 32–44 μ) and larger brochi on exine surface in *E. variegata*. In the hybrid, the dominant size class is 32 μ, and the exine surface is provided with smaller brochi as that in *E. resupinata*, and no grain of the *variegata* type has been observed to occur, which indicate the complete dominance of the characters of female pollen in the hybrid.

The present finding of the dominance of the female pollen type in the hybrid is contrary to the observations made by Hendersen[3] on *Meconopsis*, Olsson[4] on *Linaria*, Srivastava *et al.*[5] on *Amaranthus*, Srivastava[6] on *Corchorus*, and Srivastava[7] on *Tagetes*, in all of which the pollen in hybrid has been found to bear characters of the male parent. The reflection of female dominance in the pollen of *Erythrina* is substantiated by both vegetative and floral characters, and it may be presumed that it is a case of maternal inheritance. In an elaborate study of the pollen morphology of *Erythrina*, Graham and Tomb[2] recorded the occurrence of the increased abortion and multiaperturate grains as a result of hybridation, although the present study does not provide sufficient evidence in that direction.

The authors are thankful to Dr. T. N. Khoshoo, Director, National Botanic Gardens, Lucknow, for encouragement and suggestions.

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Lucknow, India, (Mrs.) KAMALESH KATIAR.
September 12, 1977. G. S. SRIVASTAVA.

---

1. Srivastava, G. S. *Queenland Garden*, 1976. 13(8), 10.
2. Graham, A. and Tomb, A. S., *Lloydia*, 1974, **37**, 465.
3. Hendersen, D. M., *Grana*, 1972, **12**(1), 52.
4. Olsson, U., *Ibid.* 1974, **14**(2 & 3) 92.
5. Srivastava, Veena, Pal, M. and Nair, P. K. K., *Rev. Palaeobot. Palynol.*, 1977, **23**, 287.
6. —, *Curr. Sci.*, 1976, **43**, 27.
7. —, *J. Palynol.*, 1976, **12**(1), 143.

REPORT OF TWO NATURAL ENEMIES OF *SPIRODELA POLYRHIZA* (L.) SCHLEID

THE menace of water weeds is reaching alarming proportions in specially tropical countries. Duckweeds are next important aquatic weeds after waterhyacinth. Amongst duckweeds, *Spirodela polyrhiza* (L.) Schleid is one of the most vigorously growing plant on the earth (National Academy of Sciences[1]). The chemical method cannot be adopted for its control because effective chemicals cause many harmful affects in aquatic ecosystem. No natural enemies of *S. polyrhiza* have been reported uptill now.

During the present study, two potent natural enemies, *viz.*, *Lymnaea luteola* f. *impura* (Troschel), a snail and *Bagous* sp., a coleoptral insect were found. They were found naturally in rare water-bodies of Gorakhpur. Both the enemies were found to show their maximum feeding capacity during January and February, which is also their maximum population period.

Lymnaea luteola f. *impura* (Troschel) is a snail of family Lymnaeidae, found in organically rich water-bodies. They were found to scrap the fleshy thallus of *S. polyrhiza*, causing permanent injury, which finally leads to distintegration of thallus very quickly. It was found that 50 starved individuals can cause death of 25 gm of *S. polyrhiza* in 7 days. *L. luteola* is also not known to serve as an intermediate host for any trematode causing diseases in human beings.

Bagous sp. is small semi-aquatic insect of family Curculionidae. The insect generally makes a small hole on the thallus of *S. polyrhiza*. Sometimes, the process is so frequent that they cause complete death of the plant. It was found that 100 individuals can cause 30% loss of fresh-weight in 7 days. The insect has been found most active during morning hours. Both the natural enemies were found to co-exist.

Thanks are due to Zoological Survey of India, Calcutta, for the identification of zoological specimen.

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1. National Academy of Sciences, *Making aquatic Weeds Useful—Some Perspectives for Developing Countries*, 1976.

PRODUCTION OF AMINO ACIDS IN *EPHEDRA FOLIATA* SUSPENSION CULTURES

THE role of amino acids in the biosynthesis of secondary plant products has been speculated[1-4]. There is little information regarding the quantitative estimation of amino acids from plant tissue cultures, although amino acids have been reported from *Datura stramonium*,[4] *D. metel*, *D. tatula*, *Momordica charantia*, *Trigonella foenum-graecum*,[5] *Ginkgo* species,[6] *Sesamum indicum*, *Tephrosia purpurea* and *T. vogelii*[7]. The present investigation deals with the production and estimation of amino acids in *Ephedra foliata* Boiss., suspension cultures.

Sterilized stem pieces of *E. foliata* were inoculated in 100 ml flasks containing 30 ml of revised[8] Murashige and Skoog's[9] medium (RT) supplemented with 1 ppm of 2, 4-dichlorophenoxyacetic acid (2, 4–4) and 1% agar. The tissue was grown and maintained for a period of twelve months as static cultures after frequent subculturings of 6–8 weeks in fresh RT medium. The static tissue was then transferred to RT liquid medium supplemented with 0·1 ppm of 2, 4–D. Suspension cultures were grown on rotary shaker for a period of five months. Three and six weeks old tissues were harvested separately and growth indices calculated (GI = Final fresh weight of tissue—Initial fresh weight of tissue/Initial fresh weight).

For the extraction of free amino acids the fresh tissue was homogenized in a Waring Blendor in 90% ethanol (1 gm/5 ml). Each of the homogenized tissue was separately centrifuged for 30 min and the residue washed three times with 90% ethanol. The supernatants were separately mixed with chloroform (1 : 3) and aqueous layer concentrated *in vacuo.*

For bound amino acids the residual tissue was hydrolysed with 7% sulphuric acid for 24 hr at room temperature. The mixture was filtered and dried under reduced pressure. Both free and bound amino acid fractions were dissolved in 50% ethanol before application.

The isolated fractions were separately applied on Whatman No. 1 filter paper strips along with 21 reference standard amino acids by using *n*-butanol–acetic acid–water; 60 : 20 : 20 in one dimension and phenol–acetic acid–water; 74 : 1 : 19·2 in the second dimension. Chromatograms were developed at 90° C for 5 min after spraying with 0·25% (wt./vol.) ninhydrin in acetone. The ninhydrin positive spots were eluted[5-7] in 90% ethanol and read in Spectronic 20 (Bausch & Lomb) colorimeter at 400 nm. Different amino acids were identified with reference to authentic samples of amino acids.

The maximum (8·0) growth index was observed in six weeks old tissue rather than three weeks old tissue (4·0). Eleven free and ten bound amino acids were detected; of them one remained unidentified (Rf 0·7). The total amino acid concentration was more in six weeks old cultures (Table I). Glutamic acid in free form and arginine in bound form were comparatively higher in concentration whereas leucine and serine had lower concentration. Arginine only in bound form and lysine and valine in free form were present.

TABLE I

Amino acid composition of Ephedra foliata *Boiss., suspension cultures*

Amino acid	Concentration (mg/g.d.w.)[a]			
	(Three weeks old)		(Six weeks old)	
	Free	Bound	Free	Bound
L-Alanine	0·62	0·35	0·90	0·83
L-Arginine	..	1·15	..	3·00
L-Aspartic acid	3·00	0·68	7·04	2·50
L-Cystine	1·94	1·74	1·20	2·94
L-Glutamic acid	1·13	0·85	7·57	2·04
Glycine	0·17	0·42	0·76	0·45
L-Leucine	1·38	0·73	0·42	0·33
L-Lycine	1·10	..	1·14	..
L-Phenylalanine	1·75	0·45	0·90	2·65
L-Serine	0·10	0·10	0·45	0·21
L-Valine	0·38	..	0·53	..
TOTAL	11·57	6·47	20·91	14·95

[a] Milligram per gram dry weight (Data based on 5 chromatographs of each sample).

Tulecke and Rutner[10] have reported that the presence of amino acids in the tissue was due to biochemical reactions at the cell surface whereas Khanna and Jain[7] and Khanna and Nag[5] reported it to be due to biochemical reaction and preferential synthesis of particular amino acid under the described conditions of cultivation of tissues. The latter view also suggests that the concentration of amino acids varies with the type and age of tissue. The wide variation in the individual and total amino acid content can be attributed to the biosynthesis of other primary and secondary products during the growth of the tissue.

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1. Chan, W. N. and Staba, E. J., *Lloydia*, 1965, **28**, 55.
2. Khanna, P. and Nag, T. N., *Ind. J. Pharm.*, 1972, **34**, 42.
3. Sairam, T. V. and Khanna, P., *Lloydia*, 1971, **34**, 170.
4. Staba, E. J. and Jindra, A., *J. Pharm. Sci.*, 1968, **57**, 701.
5. Khanna, P. and Nag, T. N., *Ind. J. Exptl. Biol.*, 1973, **11**, 310.
6. Tulecke, W., Weinstein, L. H., Rutner, A. and Laurencott, Jr. H. J., *Contr. Boyce Thompson Inst.*, 1962, **21**, 291.
7. Khanna, P. and Jain, S. C., *Ind. J. Pharm.*, 1973, **35**, 63.
8. — and Staba, E. J., *Lloydia*, 1968, **31**, 180.
9. Murashige, T. and Skoog, F., *Physiol. Plantarum*, 1962, **15**, 473.
10. Tulecke. W. and Rutner, A., *Proc. Int. Conf. on Plant Tissue Culture*, McCutchan Publ., Corp., Berkeley, California, 1965, p. 103.

ON THE OCCURRENCE OF THE NEMATODE *ASPICULURIS LAHORICA*

Aspiculuris lahorica Akhtar[1] is recorded for the first time from India. The worms were collected from the colon and the rectum part of the alimentary canal of the mouse, *Mus musculus*, at Jodhpur (Rajasthan).

A. lahorica is small, creamish white in colour, covered with thick, transversely striated cuticle. The head bears six small cephalic papillae and a cephalic bulb. A pair of cervical alae start a little behind the head-end and terminate abruptly about the middle of the oesophageal bulb. Narrow lateral alae begin a little behind the termination of cervical alae thus leaving a gap between them.

Measurements

Male (based on 5 specimens): The body is 2·95–3·70 mm long and 0·19–0·20 mm in maximum width. Cephalic bulb is 0·13×0·09 mm in diameter. the cervical alae 0·32–0·38 mm long. The oesophagus, excluding the bulb is 0·22–0·25 mm long and 0·04–0·05 mm wide. The oesophageal bulb is 0·08–0·09×0·07–0·08 mm in size. The nerve ring and the excretory pore are at 0·13 mm and 0·88–0–93 mm respectively, from the anterior end (Fig. 1).

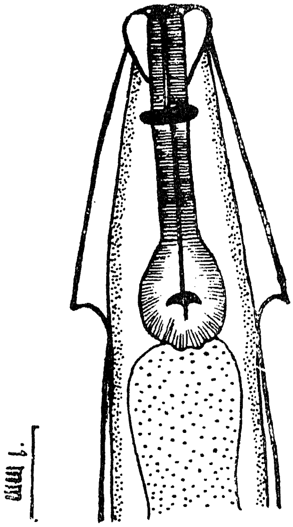

FIG. 1. Anterior end of male from a cleared toto, preparation.

The tail is 0·16 mm long with a blunt apex. There is a pair of caudal alae which extend upto the blunt apex. The caudal papillae are 10 in number—four paired and two odd ones. Of the paired papillae,–the first is preanal, the second adanal, the third post-anal and the fourth situated about the middle of the tail. Out of the two odd papillae situated in median line, the first is a little behind the posterior lip of the cloaca and the other slightly behind the former (Fig. 2).

Female (based on 5 specimens): The body is 5·00–5·40 mm long and 0·30–0·34 mm in maximum width. The cuticular striations are 0·006 mm apart. The cephalic bulb is 0·10×0·11 mm in diameter. Cervical alae are 0·43 mm. in length. The oesophagus without bulb is 0·28–0·30 mm long and 0·04–0·05 mm wide. The oesophageal bulb measures 0·14–0·15 × 0·11–0·13 mm in size. The nerve ring and the excretory pore are located at 0·16–0·18 mm and 1·00–1·14 mm respectively, from the anterior end of the body. The vulva is situated at 2·05–2·41 mm from the anterior end. The eggs are oval, 0·09×0·04 mm in size. The tail is 0·65–0·70 mm long.

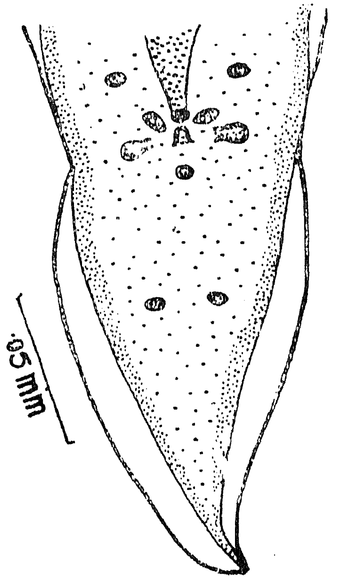

FIG. 2. Posterior end of male (Ventral view) from a cleared toto preparation.

The present specimens closely resemble Akhtar's[1], but differ from this in the location of the excretory pore and the vulva which are much posteriad in comparison to Akhtar's specimens.

The authors are very much indebted to Prof. S. D. Misra, Head, Department of Zoology, University of Jodhpur, Jodhpur, for providing the facilities.

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1. Akhtar, S. A., *Pakistan Jour. Sc. Res.*, 1955, 7, 104.

ON THE BENTHIC OSTRACODS FROM THE EAST COAST OF INDIA

THE ostracods form a well defined group of the class crustacea possessing a calcareous bivalved carapace. The geological records show that they ŏccur from the upper cambrian onwards and hence their taxonomic and stratigraphic importance. There are a few published accounts on marine planktonic[1,4,4] and benthic[2,3] ostracods from marginal water bodies. So far there is no published account of the living benthic ostracods inhabiting the brackish and marine environments from Indian waters, despite their abundance in the marginal water bodies. It is the aim of authors to explore this neglected field and to contribute to our knowledge of the ostracods from the tropical waters.

The shallow back waters of Bhimilipatnam 35 km north of Visakhapatnam and of Balacheruvu about 15 km south of Visakhapatnam which apparently provide an excellent habitat for the settlement of microfauna have been investigated by the authors for their benthic ostracods.

Regular sampling of the bottom sediment was made at monthly intervals with a devise developel by Benson[5] from October 1976 at nine prefixed stations. Data on hydrographical and sedimentological parameters were also collected simultaneously.

During the period of investigation the water temperature ranged from 24° C (December) to 37° C (May), the dissolved oxygen content from 5·6 ml/l (July) to 10·9 ml/l (February) and salinity from 15·20‰ (November) to 44·35‰ (May) in the area under investigation. Some of the common forms encountered during the survey belong to :

Family : CYTHERIDAE

Actinocytheris sp ; *Ambostracon* sp. *aff. C. packardi* (Brady 1868) ; *Carinocytheris stimpsoni* (Brady 1868). *Caudites rectangularis* (Brady 1869) ; *Campylorythere* sp. ; *Cytherella pulchara* (Brady 1880) ; *Cytherepteron alatum* (G.D. Sars 1866) *"Cythere" darwinii* (Brady 1868) ; *Illyocypris gibba* (Ramdohr 1808) ; *Loxoconcha* sp. *aff. L. sinensis* (Brady 1868) ; *Limnocythere* sp. ; *Neonesidea foveolata* (Brady 1868) ; *Tanella gracilis* (Brady 1868) and *Tanella* sps.

The preliminary studies on the shell characteristics and soft parts of the bodies of the species listed above indicate that some of the species recognised appear to be new to science. A more detailed account on the systematics and distribution of these ostracods inhabiting this area in relation to hydrographical and sedimenrological parameters will be published else where.

We are thankful to Prof. K. Hanumantha Rao, Head of the Department of Zoology, for his interest and facilities provided and to Dr. M. Subba Rao, Reader in Geology, for his interest in the study and for valuable literature furnished. One of us (C. A.) is thankful to the Andhra University for granting U.R.F. durisg the term of which this work was undertaken.

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Waltair, *October* 5, 1977.

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1. Jacob George, *Crustaceana*, 1971, 21, 141.
2. Lumbinova, P. S., Guha, D. K. and Madan Mohan, P., *India, Bull. Geol. Min. Met. Soc. India, Bull.* 22, 1960.
3. Maddock Rosalie F., *Smith Contr. Zool.*, 1969, 7, 1.
4. Merrylal James, C., *J. Mar. Biol. Ass. India*, 1973, 15, (1), 433.

4a. *Ibid*, 1973, 15 (2), 604.

5. Grossman Stauart and Benson, R. H., *Ecology*, 1967, Article I, p. 1.

STUDIES ON SHOOT BUD FORMATION *IN VITRO*

I. Effect of Immersing Embryo of *Dendrophthoe falcata* in Kinetin Solution

CYTOKININS promote shoot bud formation in a number of plant tissues cultured *in vitro*[1]. In the endosperm cultures of Taxillus vestitus Johri and Nag[2] showed that cytokinins induce, rather than enhance, bud formation. Similar induction of bud formation in the cudtures of leaf[3] and embryo[4] of *Dendrophthoe falcata* has also been reported. In these experiments the cytokinins were incorporated in the growth medium as is customary in tissue culture technique. In the present communication we describe our preliminary findings on the effect of immersing the explants in kinetin solution prior to inoculation in the tissue culture medium.

Mature embryos were dissected out aseptically from ripe fruits of *Dendrophthoe falcata* (L.f.) Ettings. and immersed in 10 ml of autoclaved kinetin solution (2 mg/ml). After immersion for 1–60 minutes, the embryos were picked up individually and inoculated on modified White's medium (WM)[5] devoid of any phytohormone. For comparison embryos were also inoculated on WM supplemented with various concentrations (2, 5 or 10 ppm) of kinetin. At least 24 cultures in test-tubes were raised for each treatment and each experiment was repeated thrice. The cultures were maintained at 25 ± 5° C in diffuse light under 50–60% relative humidity.

Shoot buds did not develop in the controls, *i.e.*, on explants inoculated either directly on WM or after being immersed in autoclaved distilled water. On the other hand, 1–15 shoot buds differentiated from the radicular end and hypocotyl of embryos immersed in kinetin solution prior to inoculation on WM (Fig. 1).

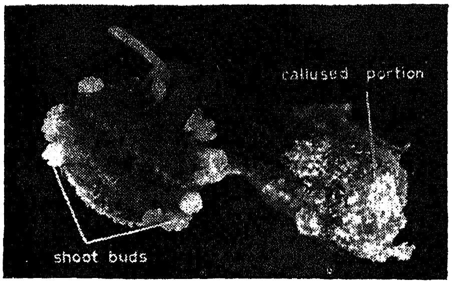

FIG. 1. *Dendrophthoe falcata* (L.f) Ettings. 4-week-old culture of embryo which was immersed in kinetin solution (2 mg/ml) for 5 minutes prior to inoculation on WM. Note the shoot buds differentiated from the radicular end and hypocotyl, × 6·2.

From embryos immersed in kinetin solution for 1–5 minutes buds appeared after about a week of inoculation. However, when they were immersed for a longer time (20, 40 or 60 min.), bud differentation occurred only after about 2 weeks. Best result was obtained by immersion for 15 min. in 2 mg/ml kinetin solution: after 4 weeks buds differentiated in about 85% cultures of embryos, and the maximum number of buds developed per explast was 17. Some of these buds grew into shoots after another 2–3 weeks. Buds also differentiated on the embryos inoculated directly on WM supplemented with kinetin (2, 5 or 10 ppm). These buds were comparable in morphology to the buds which differentiated after immersion. The minimum time required for the buds to develop in such cultures, however, was about 12 days.

It is interesting to note that kinetin can induce bud differentiation in explants immersed in it even for one minute. This shows that the action of kinetin in bud initiation is very quick. As far as we know, this is the first report of immersion of explant in the phytohormone solution prior to its inoculation in the medium of tissue culture. This technique cas profitably be utilised to study the mechanism of action of phytohormones specially during organogenesis.

We thank Professor J. P. Sinha, Head of the Department of Botany, Ranchi University, for encouragement and facilities. One of us (R. L. R.) is grateful to the UGC, New Delhi, for the award of a scholarship.

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1. Sahai Srivastava, B. L., *Int. Rev. Cytol.*, 1967, **22**, 349.
2. Johri, B. M. and Nag, K. K., *Curr. Sci.*, 1970, **39**, 177.
3. Nag, K. K. and Johri, B. M., *Planta (Berl.)*, 1970, **90**, 360.
4. — and —, *Bot. Gaz.*, 1976, **137**, 378.
5. — and —, *Phytomorphology*, 1971, **21**, 202.

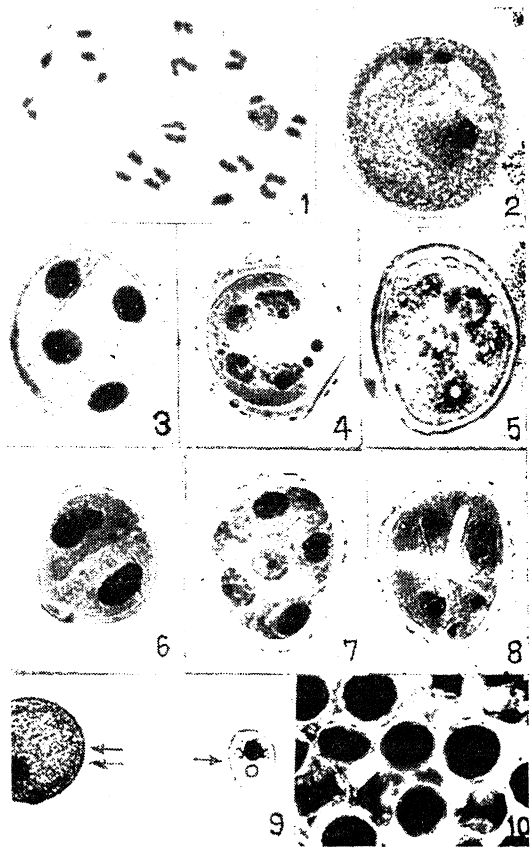
POLYMITOSIS IN THE MICROSPORES OF ELEPHANT FOOT YAM

Amorphophallus campanulatus, otherwise known as elephant foot yam, is an important subsidiary food crop found in tropics. While screening the germplasm a clone was isolated, which showed 52·0% pollen sterility while the other clones showed pollen fertility above 90%. Microsporogenesis was studied in detail to identify the causes of high pollen sterility.

The PMCs showed 14 bivalents at diakinesis (Fig. 1) and MI and later divisions were also regular resulting in normal tetrads which disjuncted into microspores. About 50% of the microspores developed in the usual way. The microspore nucleus divided into large vegetative and a small well stained generative nuclei. At the time of anthesis, the generative nucleus divided and the pollen grains became trinucleate (Fig. 2).

The remaining microspores behaved in a different manner. When the microspore nucleus divided the resultant nuclei were identical in shape and size and frequently, the second division followed immediately (Fig. 3). The division proceeded further resulting in microspores having upto six nuclei and the microspores began to show vacuolation and incipient exine could be also discernible (Fig. 4). Later, the microspore wall differentiated into distinct exine and intine (Fig. 5).

Occasionally cross wall formation took place immediately after the first division (Fig. 6) and it continued further and a number of microspores was found having as many as 5 cells (Fig. 7). Such multicelled microspores subsequently developed deep clemts (Fig. 8) and the individual cells get separated. The occurrence of micropollen with single nuclei along with normal pollen grains in the mature anthers (Fig. 9) further demonstrated that they were the product after the liberation from the multicelled microspores. In the mature anthers the pollen fertility was found to be only 48% (Fig. 10).



FIGS. 1–10. 1. Diakinesis with 14 bivalents, × 800. 2. Normal pollen with 3 nuclei, × 240. 3. Young microspore with 4 nuclei, × 400. 4. Microspore with incipient exine and showing 4 nuclei and vacuolation. × 400. 5. Pollen with 5 nuclei, × 400. 6. Young microspore with a cross wall, × 400. 7. Microspore with 5 cells, × 400. 8. Multicelled microspore showing deep clefts among the cells, × 400. 9. Normal pollen (⇇) and micropollen (→), × 150. 10. Fertile and sterile pollen, × 90.

Supernumerary divisions in the microsporocytes have been widely reported in a number of plant species[2-4] and as a consequence of these divisions the microsporocytes could produce groups on 16–20 cells. Pollen grains having eight nuclei and closely resembling the embryo sacs and known as 'Nemec Pollen' are known to occur in a few plants under special conditions and attributed the origin of abnormality to the duplication of generative nuclei[3,5]. Though, the multinucleate pollen observed in the present study did not resemble in any way with the embryo sac, the phenomenon of multicellular microspores and consequent liberation of micropollen from them is rather interesting and being reported for the first time.

The polymitosis in the elephant foot yam represent an interpolation of events not represented in its normal life history and attributed to the absence of some regulatory factor controlling the nature and stage of divisions as suggested in maize[2]. The polymitotic character of maize has been shown to be due to a recessive mendelian gene[1]. The occurrence of polymitosis in about half of the microspores makes one to suspect, the plant itself is heterozygous for this attribute and consequent of meiosis and segregation in PMC's half the microspores developed into normal pollen grains while the remaining half of the microspores which received the recessive gene responsible for polymitosis became polymitotic because it found immediate expression due to the haploid status of microspores.

The role of tapetum in the resulting partial pollen sterility can be ruled out because both fertile and sterile pollen were present inside the same anther sacs. Hence the 52% pollen sterility encountered in the present clone is due to the occurrence of polymitosis in about half of the microspores.

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---

1. Beadle, G. W., *Science*, 1929, **70**, 406.
2. —, *Cornell Univ. Agr. Expt. Sta, Mem.*, 1931, **135**, 1.
3. Geitler, L., *Ber deutsch. bot. Gesell.*, 1941, **59**, 419.
4. Kaul, A. K., *Genetica*, 1970, **41**, 305.
5. Maheshwari, P., *An Introduction to the Embryology of Angiosperms*, McGraw-Hill Book Co., London, 1950.

COCHLIOBOLUS NODULOSUS, A NEW RECORD TO INDIA

A FUNGUS with black fruiting bodies was found by the authors on dry culms, leaves and leaf sheaths of *Eragrostis pilosa* (Linn.) Beauv. during September–October, 1974. The fungus was identified as *Cochliobolus noduosus* Luttrell which has not been reported previously from India on this host. However, it has been reported by Luttrell[1] from Georgia on *Eleusine* sp. Isolation was made on PDA. Numerous pseudothecial bodies of the fungas developed after 10 days, incubation, measuring 155·10–564·00 μ × 183·30–705·00 μ. Ostiolar beak 78·70–141·00 μ × 169·00–282·62 μ;

asci 124·80–170·60 μ × 12·48–18·72 μ, 7-spored; ascospores 88·82–157·56 μ × 4·68–6·24 μ, 7 to 10 septate (mostly 9 septate), germinating from all the cells.

The culture and slides of the fungus (containing pseudothecia, asci and ascospores) have been deposited in the CMI, Kew, Surrey (No. IMI 206845) and the organism has been identified as *Cochliobolus nodulosus* Luttrell. This is a new host record and new report from India.

The authors wish to express their gratefulness to Mr. A. Johnston, Director, CMI, Ferry Lane, Kew, Surrey, for confirming the identification of the fungus and Dr. P. N. Bajpai, Head, Department of Horticulture of this University, for providing facilities. The senior author is also thankful to C.S.I.R., New Delhi, for financial assistance.

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Kanpur 208 002,
July 16, 1977.

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Luttrell, E. S., *Phytopathology*, 1957, 47, 540.

THREE NEW LEAF-SPOT DISEASES OF *KALANCHOE* SPP.

DURING July, September 1975 and 1976 three new leaf-spot diseases of *Kalanchoe* spp. were recorded at Jabalpur. All the three diseases have not so far been recorded from India.

Leaf-spot of Kalanchoe mortagei

Diseased leaves showed water-soaked, sunken, greyish, circular spots. Gradually they increased in size and the centre of the spots assumed greyish brown colour while the peripheral region became dark brown in colour. Healthy and diseased areas of the lamina were well demarcated. Sometimes many smaller spots coalesced to form bigger spots. The infected portion finally dried up and then very minute pinkish fruiting bodies were seen on the surface of lamina. The causal organism was identified as *Colletotrichum* state of *Glomerella cingulata* Spauld. & v. Schr.

The specimen has been deposited at the Herb. IMI, Kew, No. 189290 *a*, also in HPP, AUJ, No. 124.

Leaf-spot of Kalanchoe pinnata

At first small pinhead sized water soaked spots were formed on the upper surface of the leaves. The spots gradually increased in size and became amphigenous. The mature spots had a circular outline greyish brown in colour. There was a sharp demarcation between the healthy and diseased portion of the leaf. The demarcation was more pronounced on account of the presence of a narrow but distinct dark coloured raised ring. The spots may coalesce and form larger ones. At later stages, the spotted areas of the leaves became thin, dry and membranous, and very minute black fruiting bodies were clearly visible. In many cases this dried portion got detached from the healthy tissues. Isolations from the diseased leaves constantly yielded *Bartalinia robillardoides* Tassi.

The specimen has been deposited at Herb, IMI, Kew, No. 189292, also in HPP, AUJ., No. 125.

Leaf-spot of Kalanchoe fedischenkoi

Water-soaked, grey coloured spots, gradually increased in size covering the entire leaf. The cream coloured fruiting bodies were arranged in rings amphigenously. Later the diseased tissues got separated from healthy parts leaving a shothole. In severe infections the leaves shed off.

The pathogen responsible for this disease was identified as *Myrothecium roridum* Tode ex Fr.

The specimen has been deposited in HPP, AUJ, No. 126.

Pathogenicity tests were carried out in the case of all the three fungi by spraying an aqueous suspension of conidia from 10–15 days old culture on PDA with suitable controls. The disease symptoms on respective host appeared within 7–10 days. The same pathogen was obtained on reisolation in each case.

The authors express their thanks to Mr. A. Johnston, Director, Drs. M. B. Ellis and E. Punithalingam of C.M.I., Kew, for their help in the confirmation of the species.

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July 2, 1977.

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INVESTIGATIONS INTO THE CAUSES OF CHURDA MURDA (MALFORMATION) DISEASE OF CHILLI IN VIDARBHA

CHILLI (*Capsicum annuun* L.) grown in the Vidarbha region of the Maharashtra State suffers annually from a disease called 'malformation'. The disease is manifested as general stunting, reduced leaf size and upward or downward curling of leaves. Such plants also show mosaic-mottling, especially during the months August–September. Symptoms of this type being caused by varied agencies, *viz.*, (*a*) tobacco lead curl virus[1,4,5], (*b*) infestation of thrips and mites[1] and (*c*) mosaic viruses[2,3,6]. An attempt was therefore made to identify the casual complex responsible for the malformation syndrome in chilli, as it occurs in the Vidarbha region of the Maharashtra State.

Malformation syndrome consisted of three types of foliar symptoms occurring singly or in combination, *viz.*, (1) upward curling and crinckling, (2) downward curling, (3) Mottling, crinkling and puckering, (4) combination effect of 1–3 (Fig. 1). The upward curling was due to infestation of thrips (*Anaphothrips dorsalis* Hood.) and the downward curling was due to white mites (*Hemitarsonamus latus* Blank). Foliar sprays with monocrotophos (1·5 ml/l) helped such plants to regain healthy new growth.

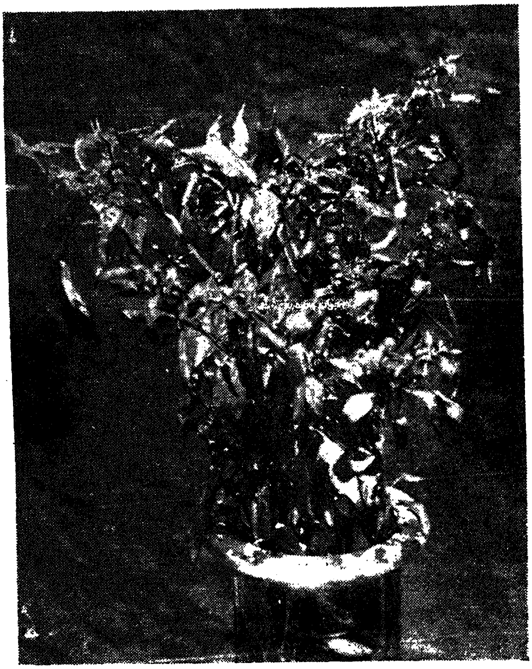

FIG. 1. Chilli plant showing 'Malformation' syndrome.

A few plants, which failed to recover even after rigorous spraying with the insecticide, harboured a viral infection. The virus was serologically related to CMV–1 as evidenced from chloroplast agglutination test. The virus in the leaf extract was sap transmissible to *Citrullus vulgaris* L. and *Datura metel* L. causing mild systemic mosaic in 3–4 weeks, and chlorotic, local lesions in 3–4 days respectively. Transmission by rub-inoculation between chillies, seemed to take a longer time. Seed transmission was negative. The virus was found to get transmitted to healthy chilli sedlings in 10–15 days, when the latter were caged along with malformed plants harbouring thrips, mites and the virus. This aspect is being verified further for obtaining direct evidence.

A feeler trial conducted in the field indicated that foliar sprays with 'Nuvocron' (Monocrotophos), 1·5 ml + 'Cuman L' (Ziram), 2 ml/l of water given at 10 days intervals effectively improved growth and productivity of NP46–A cultivar of chilli by arresting the development of malformation syndrome.

The problem of 'Churda Murda' or malformation disease of chillies, as it occurs in Vidarbha region, has thus been identified. The casual complex consists of infestation due to thrips and mites accompanied at times by infection due to a CMV–1 related mosaic virus. The virus can be detected in chilli plants by using *Datura metel* L. as a local lesion host.

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Akola (Maharashtra State),
June 22, 1977.

1. Capoor, S. P., *I.C.A.R. Tech. Bulletin No.* 12, 1967, p. 7.
2. Karma, B. S. and Dubey, G. S., *Indian Phytopath.*, 1975, **28**, 272.
3. Mathur, S. B., Mishra, M. D. and Tiwari, V. P., *Plant Dis. Reptr.*, 1966, **50**, 619.
4. Mishra, M. D., Raychaudhuri, S. P. and Jha, A., *Indian J. Microbiol.*, 1963, **3**, 73.
5. Mayee, C. D., Kaul, D. L. and Thakur, M. R., *PflaKrankh*, 1975, **10**, 566.
6. Thornberry, H. H., *U.S.D.A., A.R.S., Agric., Handbook*, 1966, No. **307**, 148.

QUALITY OF CINNAMONS MARKETED IN INDIA

ALTHOUGH true cinnamon derived from the bark of the plant *Cinnamomum zeylanicum* is admitted to be superior in flavour to cassia derived from *C. aromaticum*, *C. burmanni* or *C. loureirii*, very little of the true cinnamon is being marketed in India. Indigenous production is very little and the imports from Sri Lanka, the biggest producer (4,500 tons per annum) of this spice, is negligible[1]. Cassia is being imported from China, Indonesia and Malaysia to the tune of 50 to 60 tons annually, and sold at exorbitant prices. There is a large export of 'Indian cassia' obtained from the forests of North-Eastern India to the tune of 500–600 tons and some of this finds its way into the Indian market also. This is supposedly derived from the plant *C. tamala* whose leaves are dried and sold as the spice, *tejpatra*. But the wild trees in Assam and Manipur, from which Indian cassia is obtained, do not bear much resemblance to *C. tamala*. Indian cassia is poor in flavour. Some wild cinnamon from *C. iners* and *C. obtusifolium* and barks dipped in cinnamaldehyde are also sold by wayside vendors as *'dalchini'* or cinnamon. All this, leaves the consumer confused regarding the true cinnamon. Macroscopic and microscopic examination

TABLE I

Properties of cinnamons available in the Indian market

Popular name	Botanical origin	Properties	Yield of volatile oil %	TLC profile	GLC profile
Dalchini	*C. zeylanicum*	Pale brownish yellow quills made up of concentric tubes of thin inner bark, mild aroma and sweetish pungent taste	0·8 to 1·0	7 spots including cinnamaldehyde and eugenol	14–15 peaks including cinnamaldehyde and eugenol
Chini dalchini or Taj	*C. aromaticum* or *C. burmanni* or *C. loureirii*	Thin coarse surfaced bark of strong aroma and sweet pungent taste. Slightly mucilagenous	1 to 1·6	4 spots including cinnamaldehyde, eugenol absent	8–9 peaks including cinnamaldehyde, eugenol absent
Indian cassia	*C. tamala* ?	Thick coarse bark, highly mucilagenous, mild terpenic aroma and taste	0·5 to 0·9	4 spots including cinnamaldehyde, eugenol absent	10–12 peaks including cinnamaldehyde, eugenol absent
Jungli dalchini	*C. iners* or *C. obtusifolium*	Coarse mucilagenous bark, aroma and taste medicinal rather than cinnamon like	0·5 to 0·8	3 spots. Cinnamaldehyde and eugenol absent	5–6 peaks, cinnamaldehyde and engenol absent
Soaked bark	..	Astringent and sweet bark of strong odour	2·3 to 2·5	Only one spot of cinnamaldehyde	Only one peak of cinnamaldehyde

of the bark and thin layer and gas chromatographic examination of the volatile oils have been used before, for studying differences in cinnamon varieties[2-4].

A large number of samples of cinnamons marketed in various parts of India were obtained and analysed for the constituents, to establish the characteristic differences. The main properties of the varieties are summarised in Table I. TLC and GLC profiles of the volatile oils from the barks were markedly different.

There is need to standardise the varieties of cinnamon being sold in the country, and prevent sale of spurious and inferior barks as cinnamon.

Details of these investigations are being published elsewhere.

Plantation Products and
Flavour Technology
Discipline,
Central Food Technological
Research Institute, Mysore,
September 8, 1977.

Y. S. LEWIS.
N. KRISHNAMURTHY.
S. SHIVASHANKAR.
C. P. NATARAJAN.

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1. *Foreign Trade of India*, Director General of Commercial Intelligence and Statistics, 1976.
2. Rai Chaudhuri, H. N. and Kayal, R. N., *Bull. Bot. Sur. India*, 1971, 13, 94.
3. Lawrence, B. M., *J. Canad. Inst. Food Technol.*, 1969, 2, 178.
4. Wijesekera, R. O. B., Jayawardena, A. L. and Rajapakse, L. S., *J. Food Agric.*, 1974, 25, 1211.

REVIEWS

Electromagnetism, Electron Theory and Special Theory of Relativity. By A. K. Dutta. (The Macmillan Company of India, Ltd., 2/10 Ansari Road, Daryaganj, New Delhi 110 002), 1977. Pp. x + 331. Price Rs. 20.

The aim of the book is presumably to provide a standard, reasonably priced textbook to introduce electromagnetism and the special theory of relativity to Indian college students. The present book, however, fails to meet the elementary requirements of a student trying to learn the subject, let alone make the subject exciting and lively, The selection of the subject-matter, the arrangement of the subject-matter, the style of presentation, problems, notation, etc., leave much to be desired. The general style of the author is to provide fairly conventional, often lengthy, algebraic derivations of well-known formulae and equations, very seldom pausing to provide physical insight into the physics of the situation. Precision in presentation is one of the things which could have made such a style somewhat tolerable. Unfortunately the author often fails to define symbols as they are used. For example in Chapter 7 on electromagnetic waves, frequency of the wave is never defined. The notation used is confusing at several places; same symbols are used for different things in different chapters. Use of vector analysis is claimed to be one of the strengths of the book, yet the student is never introduced to a simple concept like the wave-vector. Instead, direction cosines and co-ordinates are used to express a plane wave. The dot-product signs are missing in a large number of equations, for example in Appendix II and in Chapter 6; something that can give nightmares to a beginner in vector analysis. The concept of a line integral and surface integral is never adequately explained, even in the Appendix. As an example of sloppiness pervading the book; in Appendix II. page 313, one reads : $d\vec{a} = \frac{d}{\rho}\vec{n}$, where ....$\vec{n}$ is the unit vector normally to $d\vec{a}$". The book seems never to have gone through a proof-reading stage.

The Indian student definitely deserves a better deal!

RAJENDRA BHANDARI.

Annual Review of Plant Physiology, Vol. 28. Edited by Winslow R. Briggs, Paul B. Green and Russell L. Jones. (Annual Reviews, Inc., 4139 El. Camino Way, Palo Alto, California 94306, U.S.A.), 1977. Pp. 615. Price: $ 17.00 in U.S.A.; $ 17.50 elsewhere.

In this review, under Cell Organisation R. F. Evert has reviewed the topic Phloem Structure and Histochemistry. Under Nutrition Absorption and Transport two topics, Mitochondrial Metabolite Transport by J. T. Wiskich and Ion Transport into the Xylem by M. G. Pitman have been reviewed. Photosynthetic Cytochromes by W. A. Cramer and J. Whitmarsh, Caynide–Resistant Respiration in Higher Plants by T. Solomos, Membrane Potentials in Photosynthesis by W. Junge have been reviewed under Bioenergetics. Four topics : Amino Acid Metabolism by B. J. Miflin and P. J. Lea, Ribulose-1-5-Biphosphate-Carboxylase-oxygenase by R. G. Jensen and J. T. Bahr, Secondary Compounds as Protective Agents by T. Swain and Regulation of Ribonucleic acid Metabolism by Plant Hormones by J. V. Jacobson have been reviewed under Metabolism. In the area of Development, six papers, (1) The Current Status of Cyclic AMP in Higher Plants by N. Amrhein, (2) Phytochromes : Membranes as Possible Sites of Primary Action by Marme, (3) Phytochrome Control of Enzymes by P. Schopfer, (4) Dormancy in Seeds by R. B. Taylorson and S. B. Hendricks, (5) Expression of Chloroplast Genomes in Higher Plants by S. Kung and (6) The Polar Transport of Auxin by M. H. M. Goldsmith are reviewed.

The Mechanism of Salt Tolerance in Halophytes by T. J. Flowers, P. F. Troke and A. R. Yeo, Environmental Interaction and the Genetics of Flowering by I. C. Murfet, and Comparative Photosynthesis of Sun and Shade Plants by N. K. Boardman are the topics reviewed in the area of environmental physiology.

Under special topics, Reaction Wood : Induction and Mechanical Action by B. F. Wilson and B. R. Archer has been reviewed.

In the prefatory chapter the paper by E. Bünning entitled, Fifty years of research in the wake of Wilhelm Pfeffer' traces the development of plant physiological research in Germany particularly the influence of earlier thinkers on experimental sciences makes interesting reading.

K. S. KRISHNA SASTRY.

6794/77. Published by Prof. M. R. A. Rao, Current Science Association, Bangalore-560 006
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IONOSPHERIC E REGION DRIFT PATTERN AT LOW-LATITUDES

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ABSTRACT

Ionospheric E region drift measurements at Ahmedabad during the period 1970–75 show a consistent pattern. Drift direction is different during winter and summer with change over in equinoxes. This agrees with the atmospheric circulation pattern obtained for the IGY period when large number of drift stations existed.

INTRODUCTION

DRIFT motion of ionospheric irregularities by spaced receiver method has been studied at Ahmedabad for different periods since 1954. The results for the period 1956–66, summarised by Rastogi[5] indicate significant seasonal and solar cycle variations. When similar measurements were made at Thumba near the magnetic equator, a very consistent picture emerged independent of season or solar cycle with drift direction always towards west by the day and towards east by the night (Rastogi *et al.*[6]). The observations at Ahmedabad were restarted in October, 1970 as part of a programme to establish a chain of drift stations in India and continued till June, 1975 This is the longest continuous spell of observations conducted at Ahmedabad and various investigations on the features of drift are being done. In this short note we present the consistent pattern of the drift observed at Ahmedabad derived from over five thousand observations during daytime (0800–1600 hr 75° E.M.T.).

RESULTS

The annual mean histogram of the percentage occurrence of drift direction for the period 0800–1600 hr local time (75° E.M.T.) for the year 1974 is shown in Fig. 1. There is no single drift direction at this latitude when the mean of such a long period is taken, although broad peaks are evident at SE and SW directions. This could be due to large seasonal variation in the pattern and so separate monthly histograms were studied. In Fig. 2, are shown the histograms of drift direction for every month averaged over the five-year period.

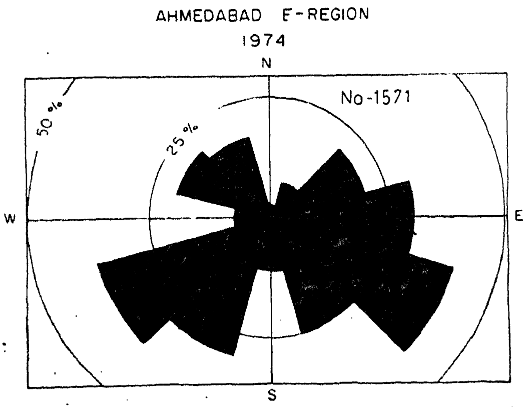

FIG. 1. Annual mean histogram of percentage occurrence of the E region drift direction at Ahmedabad during the year 1974.

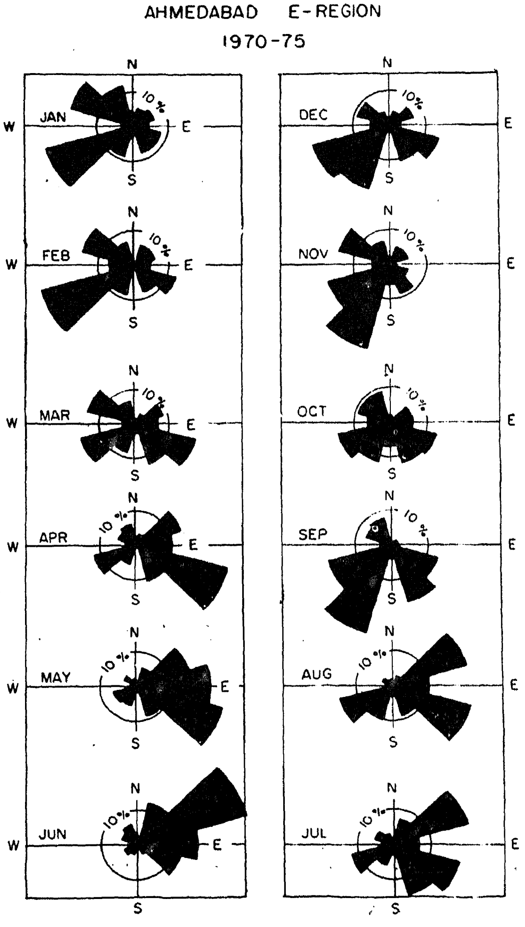

FIG. 2. Monthly mean histograms of percentage occurrence of the E region drift direction at Ahmedabad for each of the months averaged over the years 1970 to 1975.

The drift direction is mainly towards west during January with nearly equal peaks along N–W and S–W directions. The occurrence of eastward direction is very small. The eastward component increases progressively as seen from the histograms for subsequent months February, March, etc., until June when almost all occurrences are towards east. From July onwards the percentage occurrence towards east shows a decrease and westward directions increase in the subsequent months till November when again westward direction is the dominant one. The transition of the zonal component is seen during March–April and then during September and October. Rastogi[5] had earlier reported change in the drift pattern from high sunspot epoch to low sunspot epoch also. This period from October 1970 to June 1975 is primarily a low sunspot epoch and no such change has been noticed.

Discussion

Fairly lagre number of drift stations had operated during IGY–IGC and Kazimiroskiy[3,4] presented world maps of the drift vector during winter and summer. From this he could give a circulation pattern in the ionosphere. For the E region altitudes the circulation patterns were different for winter and summer. During equinoxes there was instability in the drift direction. Even though there were quite a few stations at high latitudes (with large longitude differences) the stations at low latitudes were very few.

Based on meteor winds and rocket measurements Groves[1] has computed wind model giving mean wind as a function of height and latitude for the region between 60 and 130 km. This model predicts mean wind of about 12 m/sec towards west during January and about 25 m/sec towards east during summer for a latitude of Ahmedabad considering mean E region altitude of 100 km. The data presented here are only for the daytime (0800–1600 hr) and it is not possible to compute mean winds but from earlier results for Ahmedabad (Rastogi[5]) one can find the reversal of mean zonal wind flow from westward during winter to eastward towards summer. Mean zonal flow at Udaipur, 200 km from Ahmedabad, also shows similar reversals with mean flow of about 10 m/sec towards west during winter and about 20 m/sec towards east during summer (Janve *et al.*[2]).

Acknowledgements

Thanks are due to our colleagues specially K. P. Singhal, H. O. Vats and G. D. Vyas, for their assistance in the data collection at different stages of the work. The research project is financed by the Department of Space, Government of India.

1. Groves, G. V., *J. Br. Interplanet. Soc.*, 1969, 22, 285.
2. Janve, A. V., Kumar, V., Sharma, R. V. and Rai, R. K., *Ind. J. Rad. Space Phys.*, 1977, 6, 128.
3. Kazimiroskiy, E. S., *Geomagn. Aeronomy*, 1962, 2, 897.
4. —, *Ibid.*, 1963, 3, 380.
5. Rastogi, R. G., *J. Inst. Telecom. Engrs.* 1969, 15, 216.
6. —, Chandra, H. and Misra, R. K., *Space Research*, 1972, 12, 983.

SYNTHESIS AND STRUCTURAL STUDIES OF MALONYL DIACETONE HYDRAZONE COMPLEXES OF Mn(II), Fe(II), Co(II), Ni(II), Cu(II) AND Zn(II)

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Abstract

Adducts and deprotonated complexes of the empirical compositions M(MDAH)Cl$_2$ and M(MDAH–2H)(H$_2$O)$_2$ where M = Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) and MDAH = malonyl diacetone hydrozone, have been prepared and characterized by analytical data, molar conductance, magnetic susceptibility, infrared and electronic spectral measurements.

Introduction

In continuation of our earlier work[1] on oxovanadium(IV) complexes of diacetone hydrazones, we report here the results of our studies on Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes of MDAH.

Experimental

The adducts of the type M(MDAH)Cl$_2$ were prepared by mixing together cold/hot ethanolic solutions of the metal chloride and MDAH in Ca 1 : 1 molar ratio. The deprotonated complexes M(MDAH–2H) (H$_2$O)$_2$ were prepared by mixing the aqueous solutions of the metal chloride and MDAH in Ca 1 : 2 molar ratio and raising the pH of the solution to Ca 8 by dilute sodium hydroxide solution. The complexes thus obtained were suction filtered, washed with ethanol and ether in the case of the adducts and with dilute acetic acid, water and ethanol successively in the case of deprotonated complexes and finally dried at room temperature. Experimental details pertaining to the analysis of the complexes, molar conductance, magnetic susceptibility, i.r. and electronic spectral measurements were the same as described in our earlier papers[2,3]. The analytical data, magnetic moment values and electronic bands are given in Table I.

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Results and Discussion

The deprotonated complexes lose water molecules completely in the temperature range 130–160° yielding anhydrous complexes. The loss of water molecules at high temperature and the presence of a band characteristic of coordinated[4] water in 785–810 cm^{-1} region show clearly that the water molecules are coordinated in these complexes. All the complexes do not melt up to 250°. The complexes are all insoluble in water and common organic solvents but the adducts are fairly soluble and the deprotonated complexes slightly soluble in hot DMSO. The nonelectrolytic[5] nature of the adducts is shown by the molar conductance values lying in the range 3·10–7·70 ohm^{-1} cm^{2}/mole in ·001 M DMSO solution.

Magnetic Susceptibility Measurements

The magnetic moments[6] at room temperature (Table I) indicate spin-free octahedral geometry for all Fe(II), Co(II) and Ni(II) complexes and spin-free tetrahedral or octahedral geometry for Mn(II) complexes. The μ_{eff}[7] values of Cu(II) complexes correspond to one unpaired electron from

TABLE I

Analytical data and magnetic moments of MDAH complexes

Complex	Colour	Metal %	**Chloride/ Nitrogen %	Hydrazine %	Loss of wt. %	μ_{eff} M.B.	Electronic spectral bands (nm)
Mn(MDAH)Cl_2	White	16·04 (16·26)	20·92 (21·01)	18·50 (18·94)	...	6·06	...
Fe(MDAH)Cl_2	Dirty Yellow	16·62 (16·48)	20·75 (20·96)	18·67 (18·89)	..	5·52	925
Co(MDAH)Cl_2	Pink	17·00 (17·24)	20·70 (20·77)	18·57 (18·72)	..	5·15	687, 537
Ni(MDAH)Cl_2	Green	17·15 (17·18)	20·75 (20·78)	18·75 (18·73)	...	3·38	1125, 675, 412
Cu(MDAH)Cl_2	Green	18·10 (18·36)	20·30 (20·49)	18·20 (18·47)	..	2·06	725
Zn(MDAH)Cl_2	White	18·38 (18·77)	20·25 (20·38)	18·15 (18·38)	..	Diamagnetic	..
Mn(MDAH-2H) $(H_2O)_2$	Light Yellow	18·35 (18·26)	18·55 (18·61)	21·50 (21·27)	12·45 (11·96)	6·12	..
Co(MDAH–2H) $(H_2O)_2$	Dirty Pink	19·20 (19·33)	18·30 (18·37)	21·15 (20·99)	12·45 (11·80)	5·02, 5·40*	1200
Ni(MDAH–2H) $(H_2O)_2$	Dirty Blue	19·30 (19·27)	18·25 (18·38)	20·80 (21·00)	11·50 (11·81)	3·30, 3·26*	1025, 365
Cu(MDAH-2H) $(H_2O)_2$	Dirty green	20·18 (20·53)	18·20 (18·09)	20·40 (20·67)	10·95 (11·63)	2·01	625
Zn(MDAH–2H) $(H_2O)_2$	White	20·76 (21·00)	17·80 (17·99)	20·78 (20·56)	12·05 (11·56)	Diamagnetic	..

Calculated values are given in parenthesis.

* Magnetic moments are for anhydrous complexes.

** Values in fourth column are for chloride in the case of adducts and of nitrogen in the case of deprotonated omplexes.

which it is not possible to say anything about the stereochemistry.

Electronic Spectra

The number and position of *d–d* transition bands (Table I) in the spectra of all Fe(II), Co(II) and Ni(II) complexes are consistent with the octahedral geometry[8-10]. Also, the ν_2/ν_1 ratio (1·66 for adduct and 1·53 for anhydrous deprotonated complex) for the Ni(II) complexes lies in the range reported for octahedral nickel(II) complexes[11]. The bands observed in the spectra of Cu(II) adduct and deprotonated complex are considerably blue shifted compared to the first band maxima of corresponding Ni(II) complexes owing to Jahn–Teller distortion of ^2Eg state. The distortion[12] in the Cu(II) complexes is measured by ν_{Cu}/ν_{Ni} (cm^{-1}) ratio which comes out 1·55 for adduct and 1·64 for deprotonated complex. The various ligand field parameters such as 10Dq, B', β, $\beta°$ and LFSE have been calculated[13] and found to lie in the range 7790–16000 cm^{-1}, 803–942 cm^{-1}, 0·760–0·892, 10·79–23·95% and 51·5–142·9 kJ/mole respectively. The 10Dq values of the Co(II) and Ni(II) complexes give a place between water and ammonia for MDAH in the spectrochemical series whereas the $\beta°$ values indicate the considerable overlapping of metal-ligand orbitals in the complexes.

Infrared Spectra

The bands appearing in the acetonitrile solution spectrum of the ligand at 1690, 1640, 1540, 1275 and 890 cm^{-1} are assigned[1] to amide I, ν(C=N), amide II, amide III and ν(N–N) respectively. In the spectra of the adducts amide I and amide II bands undergo a negative shift of 50–20 and 20–5 cm^{-1} respectively and amide III a positive shift of 50–10 cm^{-1} indicating coordination of both the carbonyl oxygens of the ligand in above complexes. The disappearance of these bands and appearance of new bands characteristic[14] of ν(NCO^-) in 1545–1575 and 1270–1320 cm^{-1} regions in the spectra of the hydrated and anhydrous deprotonated complexes; absence of original anion in these complexes and their 1 : 1 metal-ligand stoichiometry show the destruction of both the keto groups presumably, *via* enolization and bonding through the two resulting enolic oxygens. The presence of only one band due to coordinated $>C=N$ groups in the range 1585–1610 cm^{-1} shows the involvement in bonding of both the azomethine nitrogens of the ligand in adducts, and hydrated and anhydrous deprotonated complexes. The occurrence of ν(N–N) in 900–930 cm^{-1} region (characteristic of monodentate linking of $>N–N<$ moiety) in adducts and hydrated deprotonated complexes and at ~ 960 cm^{-1} in N–N bridging[15] region in anhydrous deprotonated complexes shows that only two azomethine nitrogens are involved in bonding in the former complexes and all the four nitrogens of the ligand in the latter complexes. The i.r. spectra thus indicate that MDAH behaves as a tetradentate ligand in all the complexes except anhydrous deprotonated complexes where it functions as a hexadentate ligand.

The nonligand bands occurring in 275–400, 250–365 and 225–310 cm^{-1} regions in the spectra of the complexes may be tentatively assigned to ν(M–O)[17], ν(M–Cl)[17] and ν(M–N)[16] modes respectively.

Based on chemical composition and physicochemical studies, polymeric structures having octahedrally coordinated metal centres may be tentatively proposed for the MDAH complexes.

ACKNOWLEDGEMENTS

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1. Aggarwal, R. C. and Singh, B., *Transition Met. Chem.*, 1976, 1, 275.
2. — and —, *Ibid.*, 1976, 1, 161.
3. — and —, *J. Coord. Chem.* (In press).
4. Nicholson, A. R. and Sutton, G. J., *Aust. J. Chem.*, 1969, 22, 373.
5. Geary, W. J., *Coord. Chem. Rev.*, 1971, 8, 81.
6. Figgis, B. N. and Lewis, J., *Prog. Inorg. Chem.*, 1964, 6, 197.
7. Cotton, F. A. and Wilkinson, G., *Advanced Inorganic Chemistry*, Wiley Eastern, 1976, p. 916.
8. Dunn, T. M., *Modern Coordination Chemistry*, Edited by J. Lewis and R. G. Wilkins, Interscience, N.Y., 1960, p. 290.
9. Matthews, R. W. and Walton, R. A., *Inorg. Chem.*, 1971, 10, 1433.
10. Lever, A. B. P., *Inorganic Electronic Spectroscopy*, Elsevier, Amsterdam, 1968.
11. Sacconi, L., *Transition Metal Chem.*, 1969, 4, 199.
12. Jorgensen, C. K., *Acta Chem. Scand.*, 1955, 9, 1362.
13. Lever, A. B. P., *J. Chem. Edu.*, 1968, 45, 8711.
14. Rao, C. N. R., *Chemical Applications of Infrared Spectroscopy*, Academic Press, N.Y., 1967, p. 265.
15 Aggarwal, R. C. and Narang, K. K., *Inorg. Chem. Acta*, 1973, 7, 65.
16. Okhaku, Noboru and Nakamoto, K., *Inorg. Chem.*, 1971, 10, 798.
17. Clark, R. J. H., *J. Chem. Soc.*, 1963, p. 1377.

THE CLAISEN REARRANGEMENT OF 7-CINNAMYLOXY DERIVATIVES OF 3-METHOXYFLAVONE AND 4-METHYL COUMARIN

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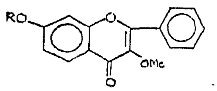
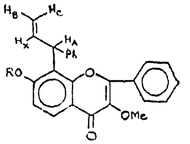
ABSTRACT

The Claisen rearrangement of 7–cinnamyloxy–3–methoxyflavone (**2**) and 4–methyl–7–cinnamyloxycoumarin (**7**) yields 8–(1–phenyl–2–propenyl)–7–hydroxy–3–methoxyflavone (**3**) and 4–methyl–8–(1–phenyl–1–propenyl)–7–hydroxycoumarin (**9**) respectively. The compound (**3**) represents the normal rearranged product and further has structural features similar to those present in some natural neoflavonoids but (**9**) is the abnormal product formed by further allylic rearrangement of the normal one (**8**).

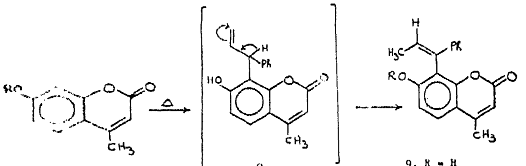
IN continuation of our work on the Claisen rearrangement of cinnamyl ethers of complex polyphenols[1,2], the rearrangement of 7-cinnamyloxy derivatives (**2** and **7**) of 3-methoxyflavone and 4-methylcoumarin respectively have been studied by refluxing them in *N, N*-dimethylaniline. In each case only one product has been isolated. Since the products are different from those reported earlier and one of them has a similar feature as is present in some natural neoflavonoids, results are reported here.



1. R = H
2. R = C_6H_5-CH=CH-CH_2-



3. R = H
4. R = Ac
5. R = Me



6. R = H
7. R = C_6H_5-CH=CH-CH_2-

8

9. R = H
10. R = Ac
11. R = Me

7-Cinnamyloxy-3-methoxyflavone (**2**) prepared from 7-hydroxy-3-methoxy-flavone[3] (**1**) by refluxing with cinnamyl bromide in the presence of potassium carbonate and acetone, crystallized from benzene-light petroleum mixture as colourless crystals, m.p. 121-22° C; R_f 0·61 (Solvent A)[1]; λ_{max} (MeOH): 219, 259 and 314 nm (log ε 4·00, 4·45 and 4·26 respectively) (Found: C, 78·5; H, 5.4. $C_{25}H_{20}O_4$ requires C, 78·1; H, 5·2%); 60 MHz NMR ($CDCl_3$): δ 3·90 (s, 3H, $-OCH_3$), 4·75 (*d*, J = 5Hz, 2H, $-OCH_2$), 6·10 - 6·50 (*m*, 2H, *CH* = *CH*), 6·73 (*br s*, 1H, *H*-8), 6·96 (*dd*, J = 10Hz and 3Hz, 1H, *H*-6), 7·16 - 8·00 (*m*, 10 *H*, 2XC_6H_5) and 8·14 ppm (*d*, J = 10Hz, 1H, *H*-5).

The above cinnamyloxy flavone (**2**) when refluxed with *N, N*-dimethylaniline gave a product which crystallised from benzene as colourless crystals, m.p. 209 - 10° C; R_f 0·25 (Solvent B); λ_{max} (MeOH): 221, 256, 316 nm (log ε 4·45, 4·39 and 4·37 respectively). Elemental analysis (Found: C, 78·5; H, 5·6. $C_{25}H_{20}O_4$ requires C, 78·1; H, 5·2%) indicated it to be an isomeric product. Its NMR spectrum showed that it has a 1-phenyl-allyl unit in the position 8. Thus its 60 MHz NMR spectrum in DMSO–d_6 showed resonance signals at δ 3·78 (*s*, 3H, $-OCH_3$), 5·11–5·27 (*m*, 1H, CH_2 = CH–$CHAr_2$), 5·30–5·65 (*m*, 2H, CH_2 = CH–), 6·23-6·83 (octet, 1H,–*CH* = CH_2), 7·08 (*d*, J = 10Hz, 1H, *H*-6), 7·17–7·36 (*m*, 10H, $-2C_6H_5$) and 7·89 ppm (*d*, J = 10Hz, 1H, *H*-5). Hence it has the structure of 8 - (1 - phenyl - 2 - propenyl) - 7 - hydroxy - 3 - methoxyflavone (**3**), in support of which it formed an acetate (**4**) and a methyl ether (**5**). The acetate (**4**) crystallised from benzene-light petroleum mixture as colourless crystals, m.p. 160-61° C; R_f 0·4 (Solvent C); 60 MHz NMR ($CDCl_3$); δ 2·12 (*s*, 3H, $-OCOCH_3$), 3·85 (*s*, 3H, OCH_3), 5·13 (*m*, J_{AX} 8·0Hz, J_{AB} = 1·6Hz, 1H, CH_2 = CH–$CHAr_2$), 5·31–5·48 (*m*, 2H, CH_2 = CH–), 6·12–6·68 (*m*, 1H, CH_2 = *CH* - CH <), 7·13 (*d*, J = 9Hz, 1H, *H*–6), 7·18 - 7·71 (*m*, 10H, 2 - C_6H_5) and 8·20 ppm (*d*, J = 9Hz, 1H, *H*-5) (Found: C, 76·2; H, 5·1, $C_{27}H_{22}O_5$ requires C, 76·0; H, 5·2%). The methyl ether (**5**) crystallised from methanol as colourless crystals, m.p. 188° C; R_f 0·6 (Solvent B); ν_{max} (nujol) 1675 cm^{-1} (> C = O); λ_{max} (MeOH) 276 and 222 nm (log ε 4·30 and 4·23 respectively) (Found; C, 78·0; H, 5·8. $C_{26}H_{22}O_4$ requires C, 78·0; H, 5·5%); 80 MHz NMR ($CDCl_3$): δ 3·83, 3·90 (2*s*. 6H 2X–OCH_3) 5·03 5·14 (*m*, 1H, CH_2 = CH–$CHAr_2$) 5·15–5·62 (*m*, 2H, CH_2 = CH–), 6·10–6·48 (*m*, 1H, CH–*CH* = CH_2) 7·05 (*d*, J = 10Hz, 1H, *H*-6), 7·12–7·46 (*m*, 10H, 2X–C_6H_5) and 8·9 ppm (*d*, J = 10Hz, 1H, *H*-5). The formation of **3** represents the normal Claisen rearrangement which is rarely met in such compounds,

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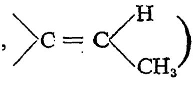
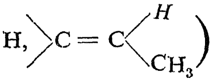
* To whom correspondence may be addressed.

This structural feature is present in some neoflavonoids such as latifolin.

4-Methyl-7-cinnamyloxycoumarin (7), prepared from 4-methyl-7-hydroxy coumarin[5] (6) in the same way as described for (2), crystallised from ethyl acetate as colourless crystals, m.p. 178–79° C, R_f 0·45 (Solvent B); λ_{max} (MeOH): 219, 253 and 320 nm (log ε 4·52, 4·57 and 4·46 respectively) (Found: C, 78·4; H, 5·5. $C_{19}H_{16}O_3$ requires C, 78·1; H, 5·5%); 60 MHz NMR (DMSO-d_6): δ 2·45 (*d*, J = 1Hz, 3H, –CH_3), 4·82 (*d*, J = 5Hz, 2H, O–CH_2–), 6·15 (*q*, J = 1Hz, 1H, *H*–3), 6·40–6·64 (*m*, 2H, Ar–*CH* = *CH*–), 6·70 (*d*, J = 2·5 Hz, 1H, *H*–8), 6·95 (*dd*, J = 10 Hz and 2·5 Hz, 1H, *H*–6), 7·20–7·40 (*m*, 5H, C_6H_5) and 7·60 ppm (*d*, J = 10Hz, 1H, *H*–5).

The above cinnamyloxy coumarin (7) on the Claisen rearrangement gave a colourless product which crystallised from ethyl acetate as colourless crystals, m.p. 222–23° C; R_f 0·6 (Solvent A); λ_{max} (MeOH): 220, 250 and 323 nm (log ε 4·54, 4·53 and 4·46 respectively). Its elemental analysis (Found: C, 78·0; H, 5·4. $C_{19}H_{16}O_3$ requires C, 78·1; H, 5·5%) showed that it is an isomeric product. The following resonance signals in its 60 MHz NMR spectrum ($CDCl_3$) established that it is 4–methyl–8–(1–phenyl–1–propenyl)–7–hydroxycoumarin (9): δ 1·68 (*d*, J = 7Hz, 3H, CH_3–CH =), 2·42 (*d*, J = 1Hz, 3H, –CH_3 in the 4 position), 6·10 (*q*, J = 1Hz, 1H, *H*–3), 6·68 (*q*, J = 7Hz, 1H,  $>C=C\begin{smallmatrix}H\\CH_3\end{smallmatrix}$) 7·00 (*d*, J=9Hz, 1H, *H*–6), 7·28 (*s*, 5H, C_6H_5) and 7·55 ppm (*d*, J = 9Hz, 1H, *H*–5). In conformity with the structure (9), it formed an acetate (10) which crystallised from ethanol as colourless crystals, m.p. 139–40° C, R_f 0·75 (Solvent B) (Found: C, 75·1; H, 5·8. $C_{21}H_{18}O_4$ requires C, 75·4; H, 5·4%) and methyl ether (11) which crystallised from methanol as colourless needles, m.p. 165° C; R_f 0·78 (Solvent B), λ_{max} (MeOH): 220, 250 and 321 nm (log ε 4·20, 4·18 and 4·14 respectively); 60 MHz NMR ($CDCl_3$): δ 1·61 (*d*, J = 7Hz, 3-H, CH_3-CH =), 2·42 (*d*, J = 1·5Hz, 3H, CH_3 in the 4 position), 3·83 (*s*, 3H, O-CH_3), 6·11 (*q*, J = 1·5Hz, 1H, *H*–3), 6·25–6·60 (*m*, 1H,  $>C=C\begin{smallmatrix}H\\CH_3\end{smallmatrix}$), 6·92 (*d*, J = 9Hz, 1H, *H*–6), 7·18 (*s*, 5H, C_6H_5) and 7·56 ppm (*d*, J = 9Hz, 1H, *H*–5).

The formation of the product (9) can be explained on the basis of the normal Claisen rearrangement followed by allylic rearrangement of the resulting intermediate (8).

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the C.S.I.R., New Delhi, for the award of JRF to DKT, and to the U.G.C., New Delhi, for the award of JRF to AK and NF to ACJ.

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1. Jain, A. C. and Gupta, R. K., *Chem. Letters*, 1974, p. 1353.
2. — and —, *Tetrahedron*, 1975, **31**, 1695.
3. Allan, I. and Robinson, R., *J. Chem. Soc.*, 1924, p. 2192.
4. R_f values are those determined on TLC plates coated with silica gel-G and solvent systems were: (A) benzene: ethyl acetate (17:3); (B) benzene: ethyl acetate (9:1); (C) benzene.
5. Pechmann, H. and Duisberg, G., *Ber. dtsch. Chem. Ges*, 1883, **16**, 2122,

THE SECOND INTERNATIONAL MYCOLOGICAL CONGRESS

The Second International Mycological Congress was held at Tampa, Florida, during September 1977 and Dr. C. V. Subramanian, Senior Professor of Botany, Madras University, Madras, was elected as the President of the Association in recognition of the research work carried by him and his associates in the field of Mycology. The following is the list of Office-bearers:

President:

Professor C. V. Subramanian, University of Madras, Madras, India.

Vice-Presidents:

Dr. S. J. Hughes, Ottawa KIA OC6, Canada; Prof. E. Muller, Zurich, Switzerland; Dr. E. Parmasto, Estonian SSR, USSR; Prof. J. Webster, Exeter, England.

Chairman:

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Secretary:

Dr. D. L. Hawksworth, Kew, England.

Treasurer:

Dr. J. A. von Arx, Baarn, Netherlands.

The Executive Committee consists of the following:

Dr. C. Booth, England; Dr. J. A. Ekundayo, Nigeria; Prof. R. Emerson, USA; Prof. K. Esser, Germany; Dr. G. Guzman, Mexico; Dr. M. V. Gorlenko, USSR; Dr. A. F. Moustafa, Kuwait; Dr. J. F. Peerally, Mauritius; Prof. H. J. Phaff, USA; Dr. L. Ryvarden, Norway; Dr. J. A. Saenz Renauld, Costa Rica; Dr. E. G. Simmons, USA; Prof. K. S. Thind, India; Dr. K. Tubaki, Japan; Dr. J. Walker, Australia and Dr. G. C. A. van der Westhuizen, South Africa.

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KINETICS AND MECHANISM OF THE HYDROLYSIS OF DI-2-PHENYLETHYL PHOSPHATE IN ACID MEDIUM

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ABSTRACT

Investigation of the hydrolysis of di-2-phenylethyl phosphate in 10% dioxan-water at 98° C in the region 1·0 M to 7·0 M hydrochloric acid shows that it is reactive mainly *via* conjugate acid species. Unlike other substituted alkyl phosphates it shows bend in higher acid region. Ionic strength data shows positive salt effect. Rate coefficients estimated from ionic strength data agree well with the experimental rates. Solvent effect, solvent–isotope effect, Arrhenius parameters and Hammett acidity function have been found to favour unimolecularity of the reaction. Comparative rate data of other esters have been used to discuss the probable reaction paths.

INTRODUCTION

THERE have been very few studies of the kinetics of hydrolysis of simple dialkyl esters. Under all conditions studied, hydrolysis predominantly occurs with the fission of C—O bond. Only dimethyl[1] and dibenzyl[2] phosphates have been studied in detail. In the present investigation, hydrolysis of di-2-phenylethyl phosphate has been studied with a view to know its behaviour in acid region and the results are presented in this paper.

MATERIALS AND METHODS

Di-2-phenylethyl phosphate was prepared by shaking 2-phenylethanol with PCl_5 in the ratio of 3 : 1. In this method triester was also formed. The diester was separated from triester by dissolving it in 10% NaOH solution and then reprecipitated by adding conc. HCl. It was recrystallised from petroleum ether (60–80). M.P. 58° C. (Found C, 62·84% ; H, 6·37% ; P, 9·80% for $C_{16}H_{19}O_4P$. Required C, 62·74% ; H, 6·20%, P, 10·13%).

Deuterium-oxide was obtained from B.A.R.C., Bombay. All the chemicals used were of B.D.H. (A.R.) quality. The kinetic runs were carried out at 98° ± 0·5° C taking 0·0005 M solution of the ester in 10% dioxan water (v/v) mixture. The rate of reaction was determined by colorimetric estimation of inorganic phosphate by Allen's method[3].

RESULT AND DISCUSSION

Pseudo-first order rate coefficients for the hydrolysis of di-2-phenylethyl phosphate in the region 1·0 M to 7·0 M hydrochloric acid show that the rate rises with rise in acidity upto 5·0 M and then decreases (Table I). The rise in rate with rise in acidity may either be due to incursion of conjugate acid species or to positive ionic strength effect on the hydrolysis *via* neutral species or may be due to both of these effects. Similar maxima have also been observed during hydrolysis of dimethyl[1] and diphenyl[4] phosphates.

TABLE I

Observed and calculated rates of the hydrolysis of di-2-phenylethyl phosphate in acid medium at 98° C

HCl	$10^5 k$ (min.$^{-1}$) (experimental)	$10^5 k$ (min.$^{-1}$) (calculated)
1·0	5·47	5·71
2·0	11·67	10·53
3·0	22·01	25·43
4·0	52·59	58·10
5·0	70·49	69·50 (*n* 1)
	75·75*	
	88·75**	
	104·00***	
6·0	64·64	62·77 (*n* 3)
7·0	50·67	42·70 (*n* 4)

Rates in * 30%, **40% and ***50% dioxan respectively.

Kinetic runs were carried out at constant ionic strength (Table II). At each ionic strength rate increases linearly with the increase in acid concentration. The dependance of rate coefficients on ionic strength is satisfactorily represented by the second emperical term of Debye-Hückel equation[5], $k' = k_0 e^{b\mu}$, where k' and k_0 are respectively the rate constants at any ionic strength and rate at zero ionic strength, b is a constant and μ, ionic strength. At any ionic strength the total rate of hydrolysis, k_e is given by the equation :

$$k_e = k_{N_0} e^{b\mu} + k_{H_0^+} \cdot C_{H^+} \cdot e^{b'\mu}$$

where k_{N_0} and $k_{H_0^+}$ are respectively the rate constants for neutral and conjugate acid species at

TABLE II

Rates of the hydrolysis of di-2-phenylethyl phosphate at constant ionic strength at 98° C

HCl (M)	NaCl (M)	10^3 *k* (min.$^{-1}$) (experimental)
	$\mu = 1 \cdot 0$	
0·20	0·80	0·023
0·40	0·60	0·025
0·60	0·40	0·034
0·80	0·20	0·044
	$\mu = 2 \cdot 0$	
0·20	1·80	0·029
0·50	1·50	0·033
1·50	0·50	0·089
1·80	0·20	0·148
	$\mu = 3 \cdot 0$	
1·00	2·00	0·084
1·50	1·50	0·123
2·00	1·00	0·158
2·50	0·50	0·189

zero ionic strength. The experimentally observed values are : $k_{H_0^+} = 0 \cdot 15 \times 10^{-5}$ min.$^{-1}$, $k_{N_0} = 1 \cdot 6 \times 10^{-5}$ min.$^{-1}$, $b = 0 \cdot 00$ and $b' = 0 \cdot 25$. Calculated rates from the above equation agree well with the experimentally observed rates (Table I). For the reaction in higher acid media (> 4 M) agreement between the calculated and experimental rates can be sought by water activity and may be represented by the equation :

$$k_e = k_{N_0} \cdot e^{b\mu} + k_{H_0^+} \cdot C_{H^+} \cdot e^{b'\mu} \cdot (a\, H_2O)^n$$

where *n* is integer whose value increased with the increase in acidity.

Kinetic data of a series of aqueous-dioxan mixtures in 5·0 M HCl at 98° C show that the rate increases with increase in dioxan content (Table I). The elevation in the rate of hydrolysis may be attributed to the dioxan, a better proton **donar**[6] in aqueous-dioxan media.

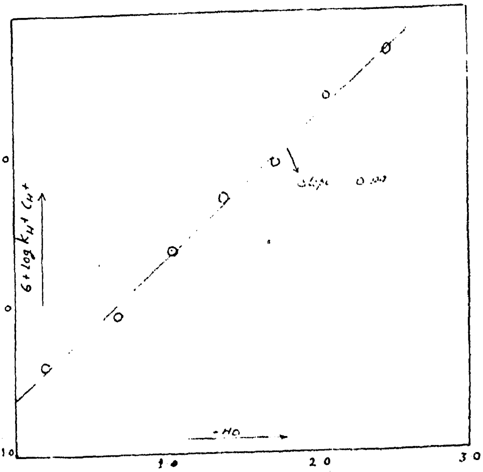


FIG. 1. Hammett plot for the hydrolysis of Di-2-phenylethyle phosphate at 98° C.

Solvent isotope effect in 3·0 M hydrochloric acid ($k_{D_2O}/k_{H_2O} = 1 \cdot 04$) favours the formation of conjugate acid species by a fast pre-equilibrium proton transfer[7].

TABLE III

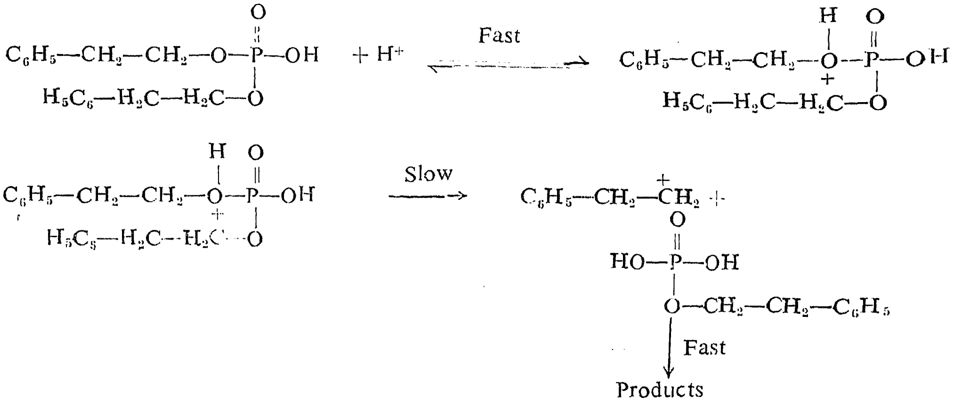
Comparative kinetic rate data for the hydrolysis of diesters via *conjugate acid species*

Phosphates	Temp. °C	E (K.cal/mole)	ΔS‡	Fission	Ref.
Di-allyl-	80	29·30	3·90	C—O	11
Di-*p*-iodo benzyl-	80	24·25	− 9·64	C—O	12
Di-*p*-nitrobenzyl-	80	18·69	−27·99	C—O	13
Di-2-phenylethyl-	98	36·59	17·94	C—O	This work
Di-methyl-	100	..	..	C—O	1

A plot of log-rate *versus* Hammett acidity function[8] (Fig. 1) shows specific acid catalysed unimolecular hydrolysis of the diester[9]. Arrhenius parameters[10] (E = 36·59 K.cal. mole^{-1}, A = $7 \cdot 638 \times 10^{17}$ sec.$^{-1}$, ΔS‡ = 17·94 e.u.) in 6·0 M HCl also support the unimolecular nature of the reaction.

Comparative kinetic rate data of other diesters (Table III) of known mechanism support unimolecular hydrolysis of di-2-phenylethyl phosphate *via* C—O bond fission.

In light of the above results the mechanism of the acid hydrolysis of di-2-phenylethyl phosphate may be formulated as :



1. Bunton, C. A., Mhala, M. M., Oldham, K. G. and Vernon, C. A., *J. Chem. Soc.*, 1960, p. 3293.
2. Kumamoto, J. and Westheimer, F. H., *J. Am. Chem. Soc.*, 1955, 77, 2515.
3. Allen, R. J. L., *Biochem. J.*, 1940, 34, 858.
4. Barnard, P. W. C., Bunton, C. A., Kellarman, D., Mhala, M. M., Silver, B. L., Vernon, C. A. and Welch, V. A., *J. Chem. Soc.*, B, 1966, p. 227.
5. Long, F. A. and McDevit, W. F., *Chem. Rev.*, 1952, 51, 119.
6. Chanley, J. D. and Feageson, E., *J. Am. Chem. Soc.*, 1958, 80, 2686.
7. Bell, R. P., *Acid Base Catalysis*, Clarendon Press, Oxford, 1949, p. 145.
8. Hammett, L. P., *Physical Organic Chemistry*, McGraw-Hill Book Co., London, 1940, p. 273.
9. Long, F. A. and Paul, M. A., *Chem. Rev.*, 1957, 57, 1.
10. Schalegar, L. L. and Long, F. A., *Advances in Physical Organic Chemistry*, Edited by V. Gold, Academic Press, Inc., London. 1963, Vol. 1.
11. Mhala, M. M. and Saxena, S. B., *Indian J. Chem.*, 1972, 10, 704.
12. Killedar, A. V., *Ph.D. Thesis*, Jiwaji University, Gwalior, 1972.
13. Kavdikar, V. M., *Ibid.*, Jiwaji University, Gwalior, 1970.

SCIENCE ACADEMY MEDALS FOR YOUNG SCIENTISTS, 1978

These medals are awarded annually by the INSA in recognition of outstanding work of scientists below the age of 30 (as reckoned on 31st December preceding the year of award). With the medal there is a cash price of Rs. 1,500/–. In addition, a research grant of Rs. 5,000/– is provided by the Kothari Scientific and Research Institute, Calcutta. Nominations may be made by Fellows of the Academy, as also by Scientific Societies, University Faculties and Departments, and Research Institutions. Members of Sectional Committees of the Academy should not sponsor candidates for the award. The last date for the receipt of nominations is January 31, 1978. Further information and nomination forms may be obtained from the Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 110 002, by sending a self-addressed envelope 28 cm × 12 cm.

CANCER DETECTION AND PREVENTION *

THE new Journal, *Cancer Detection and Prevention*, brought out by International Study Group for the Detection and Prevention of Cancer (DePCa) fills up a real need for a forum for regular and continuing discussion for the workers in preventive and clinical oncology.

Despite herculean efforts, only one out of three patients with the diagnosis of cancer, have a chance of cure. The primary prevention' of cancer by identification of environmental carcinogens, and secondary prevention' by detection of cancer by identification and screening of high risk population has increasingly come to occupy a central place in cancer control.

Dr. Selikoff discusses the methodology of search for carcinogenic environmental agents. The alert physician plays an important role in identification of etiological agents, especially if the cancer produced is uncommon ; however when the cancer produced has a high incidence and the etiologic agent rather ubiquitous in the environment, the identification remains elusive unless thorough epidemiological studies are undertaken. Creech and Johnson discovered three cases of angiosarcoma of the liver in workers at a factory using vinyl chloride, however the same chemical is used as a propellant in aerosol cans, thus a much larger group is exposed to it in smaller amount. Thus among too many leads, priorities have to be set in pursuing the most rewarding leads to achieve maximum cost-effectiveness. The existence in the West of large population groups (industrial labour, school students, social security claimants) with excellent records of their health makes epidemiological studies easy to plan and execute.

Oncogenicity of drugs is discussed by Clayson and Shubik. Several drugs with oncogenic properties have been identified, some before and some after their release for general clinical use. In modern medicine, chronically administered drugs represent one of the major exposures of man to exogenous chemicals. Analgesics, anti-rheumatic drugs, hormones and immunosuppressor drugs represent some of the classes of drugs to which an increasing segment of the population is exposed. Herbst reported the occurrence of twelve cases of hitherto rare tumour, clear cell adenocarcinoma of vagina in the daughters of women whose pregnancy has been maintained on diethylstilbesterol. The reported and confirmed increase of cancer (especially skin cancer, reticulum cell sarcoma) in long term survivors following renal transplant was initially thought to be only due to immune suppression by drugs administered to maintain the transplant, however there is some reason to believe that these chemicals may themselves be responsible for the increase in neoplasia in these patients.

The role of epidemiology in elucidating the importance of environmental factors in human cancer is discussed in masterly fashion by Higginson and Muir. Unfortunately most physicians are ignorant of the methodology of analytical epidemiological studies, and are unable to evaluate the mounting evidence supporting environmental etiology for cancer for between 70 to 90% of the cases. This evidence is essentially of two types. First group of cancers include carcinoma of lung, carcinoma of oesophagus and mesothelioma for which the equivalent of Koch's postulate in cancer epidemiology are more or less satisfied with the evidence available, though extrapolation from animal studies to man does present a problem. The second group includes those cancers where environmental stimulus remains unidentified, but available data from descriptive epidemiology, migrant studies, interpretation of time trends, corelation studies, etc., provide strong circumstantial evidence of as yet unidentified environmental agent or agents.

Kingsley discusses briefly the problem of exposure of agricultural workers to various carcinogenic chemicals. This happily is not a major problem in India where some advantages of backwardness still remain. Newberne discusses the role of environmental modifiers like drugs, chemicals and dietary factors which act to alter the individual and the response to environmental carcinogens. Lynch *et al.*, contributed a paper on the genetics of cancer resistance and susceptibility. The persons and families carrying the autosomal dominant genes for the rare forms of hereditary cancers form a group of intense interest to the cancer geneticist, but their study is less likely to contribute to cancer control and prevention as the extent of environmental control required is hardly likely to be achieved. For the present at least, the most fruitful venue is likely to be the identification of environmental carcinogens.

The Journal is published quarterly and future issues devoted to subjects as etiology, detection and diagnostic management of cancer of the breast, radiation hazards, systemic markers of early cancer, will be looked forward to by the workers in clinical as well as laboratory oncology.

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* *Cancer Detection and Prevention*. Vol. I, No. 1. Sponsored by the International Study Group for the Detection and Prevention of Cancer. (Marcel Dekker, Inc., 270, Madioar Avenue, New York, N.Y. 10016), 1976 (2 copies per Volume), Pp. iv + 239. Price : $27.50 ; add $2.30 per Volume for postage and handling outside U.S.A.

LETTERS TO THE EDITOR

X-RAY DETERMINATION OF THE MEAN AMPLITUDE OF VIBRATION AND THE DEBYE TEMPERATURE OF Gd-Zn AND Gd-Mg

GADOLINIUM forms alloys with the cubic CsCl structure with cadmium, magnesium and zinc. There is, in general, a dearth of data regarding the lattice dynamical properties of these substances. The X-ray determination of the Debye temperature and mean amplitude of vibration of Gd–Mg and Gd–Zn has been carried out in these laboratories. The results are reported here.

In each case the material was reduced to a fine powder by grinding and a flat powder specimen was prepared. Measurements were made with a Philips PW 1051 diffractometer using CuKα radiation, filtered to eliminate Kβ radiation. An argon-filled G.M. counter was used to collect the intensity data. A 2θ scan was adopted.

The peak to background ratio of the reflections recorded was low for both Gd–Zn and Gd–Mg. Only seven measurable reflections in the case of Gd–Zn and six for Gd–Mg could be recorded in the angular range possible for the diffractometer.

The lattice constant of Gd–Zn was found to be 3·600 Å. This is in good agreement with the values 3·602 Å (Chao and Duwez[1]) and 3·601 Å (Buschow[2]) reported earlier. For Gd–Mg, Chao and Duwez report a value of 3·824 Å for the lattice constant, while Kripyakevich *et al.*[3] give a value 3·79 Å. The present value 3·812 Å is intermediate between the two. It must however be noted that the lattice constants were determined from the Bragg angles of the last three reflections recorded in each case and were not corrected for systematic errors.

The intensities were corrected for temperature diffuse scattering by the method of Chipman and Paskin[4], and for dead time effects by an approximate method proposed by Chipman[5], employing a value of 150 μs for the dead time of the counting apparatus.

For a flat powder specimen, the observed intensity I_0 is given by

$$I_0 = C\,(LP)\,J\,F_T^2 \qquad (1)$$

where C is a constant, LP the Lorentz-polarisation factor and J the multiplicity factor. Due to the small number of reflections recorded, a common Debye-Waller factor was assumed for both the atoms, in each of the crystals studied. With this approximation, the structure factor F_T for a CsCl type crystal is given by

$$F_T = F e^{-B(\sin^2\theta/\lambda^2)} \qquad (2)$$

where θ is the Bragg angle, λ the wavelength of the radiation used and $F = f_A \pm f_B$. Here, f_A and f_B are the atomic scattering factors for atoms of type A and B. The plus sign refers to reflections with $(h+k+l)$ even and minus sign to reflections with $(h+k+l)$ odd.

The atomic scattering factors for Gd were taken from Cromer and Waber[6] and for Zn and Mg from those given in the *International Tables for X-ray Crystallography*[7] following a recommendation by Cromer[8]. The scattering factors were corrected for anomalous dispersion (Cromer and Liberman[9]).

The value of B can be obtained from a plot of log (I_0/I_C) against $\sin^2\theta/\lambda^2$, where $I_c = (LP)\ JF^2$. The root mean square amplitude of vibration $(\bar{u}^2)^{1/2}$ and the Debye temperature have been determined following the method described in an earlier paper[10].

Plots of log (I_0/I_C) against $\sin^2\theta/\lambda^2$ for both Gd–Zn and Gd–Mg are shown in Fig. 1. The values of the mean Debye-Waller factor B, the mean amplitude of vibration and the Debye temperature obtained are given in Table I.

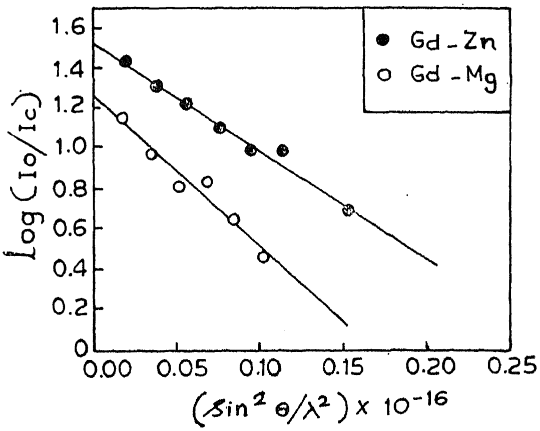

FIG. 1. Plot of log (I_0/I_C) and $\sin^2\theta/\lambda^2$ for Gd–Zn (●) and Gd–Mg (○).

There seems to be no earlier determination of the Debye temperature of either Gd–Zn or Gd–Mg by any method. The errors quoted refer only to random errors in the measurement of intensities.

From a consideration of other properties, Buschow[11] suggests that the two species of atoms exchange their sites to a certain degree. This is certain to affect the intensities. However, no information about this aspect could be obtained due to the small number of reflections recorded.

TABLE I

Values of the Debye-Waller factor B, the mean amplitude of vibration $(\bar{u}^2)^{1/2}$ *and the debye temperature* θ *at room temperature of Gd–Zn and Gd–Mg*

Substance	B (Å^2)	$(\bar{u}^2)^{1/2}$ (Å)	θ(°K)
Gd–Zn	$2 \cdot 665 \pm 0 \cdot 162$	$0 \cdot 318 \pm 0 \cdot 010$	108 ± 3
Gd–Mg	$3 \cdot 697 \pm 0 \cdot 464$	$0 \cdot 375 \pm 0 \cdot 024$	101 ± 6

Thanks are due to Dr. K. H. J. Buschow of Philips Laboratories for a gift of the samples of Gd–Zn and Gd–Mg.

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1. Chao, C. C. and Duwez, P., *J. Appl. Phys.*, 1966, **37**, 2631.
2. Buschow, K. H. J., *Ibid.*, 1973, **44**, 1817.
3. Kripyakevich, P. I., Terekhova, V. F., Zerechnyuk, O. S. and Burov, I. V., *Sov. Phys. Crystallogr.*, 1963, **8**, 203.
4. Chipman, D. R. and Paskin, A., *J. Appl. Phys.*, 1959, **30**, 1998.
5. —, *Acta Cryst.*, 1969, **A25**, 209.
6. Cromer, D. T. and Waber, J. T., *Ibid.*, 1965, **18**, 104.
7. *International Tables for X-ray Crystallography*, Kynoch Press, Birmingham, 1968, **3.**
8. Cromer, D. T., *Acta Cryst.*, 1965, **19**, 224.
9. — and Liberman, D., *J. Chem. Phys.*, 1970, **53**, 1891.
10. Subhadra, K. G. and Sirdeshmukh, D. B., *Pramāṇa*, 1977, **9**, 223.
11. Buschow, K. H. J., Private communication.

FRANCK-CONDON FACTORS AND *r*-CENTROIDS FOR THE ($A^2\Pi - X^2\Sigma^+$) TRANSITION OF CN BY LANGER'S APPROXIMATION METHOD

THE Red band System of CN ($A^2\Pi - X^2\Sigma^+$) transition is well known. Good deal of work, both experimental and theoretical, has been done on this particular system. In the present investigation we have chosen this transition for testing the validity of one of the less known methods of solving the Schrödinger wave equation approximately. This method is due to Langer[1], and very briefly it may be described as follows :

The one-dimensional Schrödinger wave equation is written as

$$\frac{d^2\psi}{dx^2} + \lambda^2 [E - V(x)]\, \psi = 0 \tag{1}$$

where $\lambda^2 = 2\mu/\hbar^2$, μ = reduced mass, $\hbar$ = Planck's constant, $V(x)$ = Potential energy function.

The variable is changed from x to z through the relation

$$Z = x - x_e - \left(\frac{\sigma}{\lambda}\right) \tag{2}$$

where

$$\sigma = -\frac{2V'''(x_e)\,\lambda\, E}{9\,[v''(x_e)]^2} \tag{3}$$

x_e = equilibrium internuclear distance; the primes indicate derivatives with respect to x. Equation (1) then becomes

$$\frac{d^2\psi}{dx^2} - [\lambda^2\psi_0{}^2(Z) + \lambda\, \psi_1(Z) + \psi_2(Z)]\, \psi = 0 \tag{4}$$

where

$$\psi_0{}^2(Z) = V(z + x_e)$$

$$\psi_1(Z) = V(z + x_e)$$

$$\psi_2(Z) = \lambda^2 \left\{ V\left(z + x_e + \frac{\sigma}{\lambda}\right) - V(z - x_e) - V'(z + x_e \right\}.$$

Finally the solutions will come out as

$$\psi_n = \left[\frac{2V''(x_e)}{\pi}\right]^{1/4} \left(\frac{n!}{2^n}\right)^{1/2} \times \frac{2(-1)^{(n-1)/2}}{\left(\frac{n-1}{2}\right)!} \frac{M_{\frac{n}{2}+\frac{1}{4},\, -\frac{1}{4}}(\xi)}{\sqrt{\phi(z)}}$$

where n is odd

$$\psi_n = \left[\frac{2V''(x_e)}{\pi}\right]^{1/4} \left(\frac{n!}{2^n}\right)^{1/2} \times \frac{(-1)^{n/2}}{(n/2)!} \frac{M_{\frac{n}{2}+\frac{1}{4},\, -\frac{1}{4}}(\xi)}{\sqrt{\phi(z)}}$$

where n is even.

where the M's are confluent hypergeometric functions.

Using these wave functions and the Rydberg potential defined by

$$V(x) = -D\,[1 + a(x - x_e)]\, e^{-a(x-x_e)}$$

where D = dissociation energy and a is a constant, we have computed wave functions for the $A^2\Pi - X^2\Sigma^+$ transition of CN and used these wave functions to evaluate Franck-Condon factors and r-centroids defined by the following relations :

$$\text{Franck-Condon factor (FC factor)} = \left[\int \psi^*_{v'} \; \psi_{v''} \; dr\right]^2$$

$$r\text{-centroid } (\bar{r}_{v'v''}) = \frac{\int \psi^*_{v'} \, \gamma \, \psi_{v''} \, dr}{\int \psi^*_{v'} \, \psi_{v''} \, dr}$$

These results have been tabulated in Table I.

TABLE I

Band	FC Langer (Rydberg potential)	FC Klein-Dunham potentials	RC $\bar{r}_{v'v''}$ Langer (Rydberg potential)	RC $\bar{r}_{v'v''}$ Klein-Dunham potentials
(0, 0)	0·490	0·488	1·212	1·206
(0, 1)	0·377	0·378	1·252	1·252
(0, 2)	0·118	0·115	1·303	1·305
(0, 3)	0·019	0·018	1·372	1·365
(1, 0)	0·333	0·325	1·164	1·165
(1, 1)	0·045	0·0414	1·221	1·223
(1, 2)	0·357	0·348	1·261	1·261
(1, 3)	0·220	0·224	1·351	1·313
(2, 0)	0·129	0·130	1·131	1·128
(2, 1)	0·250	0·237	1·185	1·173
(2, 2)	0·013	0·0125	1·194	1·189
(2, 3)	0·216	0·210	1·262	1·271
(3, 0)	0·035	0·041	1·149	1·095
(3, 1)	0·211	0·195	1·133	1·135
(3, 2)	0·105	0·0983	1·193	1·184
(3, 3)	0·097	0·089	1·215	1·214

In the same table FCs and $\bar{r}_{v'v''}$ s obtained with the use of Klein-Dunham potential by McCallum and Nicholls[2] have been given for comparison.

As one can see from the table there is very good agreement between our sets of FCs and $\bar{r}_{v'v''}$ s with those of McCallum and Nicholls at low vibrational quantum numbers. For bands with higher quantum numbers the agreement is not so good. This is as it should be, for, it is known that Langer's procedure holds good only at low quantum numbers.

The advantage of Langer's method consists in its applicability to any potential which can be expressed in closed form. Thus one could use such different analytic potentials and compare their suitability or otherwise for a given transition. Work on these lines is in progress in our laboratories.

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1. Langer, R. E., *Phys. Rev.* 1949, 75, 1573.
2. McCallum, J. C. and Nicholls, R. W., *Spectroscopic Reports, C.R.E.S.S.*, York University, Toronto, Canada, 1972.

NQR IN CERTAIN ORGANIC SOLIDS

Nuclear Quadrupole Resonance (NQR) frequency of ^{35}Cl has been measured in five organic solids, using an NQR spectrometer consisting of a frequency modulated, self-quenched superregenerative oscillator-detector. The resonance frequencies were measured with a digital frequency counter with an accuracy of ± 2 KHz.

The measured NQR frequencies for the five solids at room temperature and at 77° K are given in Table I.

TABLE I

Sl. No.	Name of the solid	^{35}Cl NQR frequency (MHz) at 77° K	at 301° K
1.	2-amino-5-chloropyridine	35·630[a]	
		35·628	35·126
2.	2, 4-dichlorobenzoic acid	35·528[b]	
		35·527	35·032
		37·432[b]	
		37·429	36·868
3.	4-chloro-2-nitrobenzoic acid	36·114	35·531
4.	5-amino-2-chlorobenzotrifluoride	36·391	35·548
5.	2-amino-5-chlorobenzotrifluoride	35·308	*

[a] Bray *et al.*[1]; [b] Bray and Barnes[2].
* The compound is a liquid at room temperature.

Nuclear quadrupole resonance has been observed for the first time in the three solids, namely, 4-chloro-2-nitrobenzoic acid, 5-amino-2-chloro-benzotrifluoride and 2-amino-5-chlorobenzotrifluoride (liquid at room temperature). For the other two solids, 2-amino-5-chloropyridine and 2, 4-dichlorobenzoic acid, the NQR frequency has been reported earlier at 77° K only by Bray *et al.*[1] and Bray and Barnes[2]. We have measured

NQR frequency in these two solids both at 77° K and at room temperature. Two resonance lines have been observed in 2, 4-dichlorobenzoic acid due to chemically inequivalent sites.

The resonance frequency of 5-amino-2-chlorobenzotrifluoride is higher than that of 2-amino-5-chlorobenzotrifluoride. This may be explained by the fact that chlorine occupying the ortho position will have a higher resonance frequency when compared to the chlorine occupying the meta position (Nagarajan and Murthy[3]). Similarly, when the resonance frequency of 4-chloro-2-nitrobenzoic acid is compared with that of 4-chloro-3-nitrobenzoic acid (37·483 MHz—Bray and Barnes[2]) and 4-chlorobenzoic acid (34·673 MHz—Meal[4]), we observe that the introduction of nitro group increases the resonance frequency. Also, 4-chloro-3-nitrobenzoic acid has an higher resonance frequency than 4-chloro-2-nitrobenzoic acid. This is because when the chlorine occupies the ortho position with respect to the nitro group a higher resonance frequency will result as compared with the chlorine in the meta position (Ref. 3).

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1. Bray, P. J., Moskowitz, S., Hooper, H. O., Barnes, R. G. and Segel, S., *J. Chem. Phys.*, 1958, **28**, 99.
2. — and Barnes, R. G., *Ibid.*, 1957, **27**, 551.
3. Nagarajan, V. and Murthy, C. R. K., *Ind. J. Pure and Appl. Phys.*, 1963, **1**, 168.
4. Meal, H. C., *J. Am. Chem. Soc.*, 1952, 74, 6121.

IN-SITU MEASUREMENT IS A MUST FOR SIZE EFFECT STUDIES OF THIN METAL FILMS*

THE anomalous behaviour of the size dependence of the electrical transport properties in metals, semimetals, semiconductors, alloys, etc., may be associated with the anomalous conditions in the structure of thin films which should depend very much on the method and environment of deposition. So the experimental conditions should be the same as those which have been postulated when deriving relations in the theory of size effects (Fuchs[1], Sondheimer[2]). It has been observed (Garcia *et al.*[3]) that the film properties change drastically when the film is removed from high vacuum. So according to them, for accurate results the measurements should be done without removing the film from the high vacuum (*i.e.*, *in-situ*). Here the resistivity values for each of silver and copper films measured under *in-situ* and not *in-situ* conditions and deposited under extremely controlled evaporation conditions have been reported. In an earlier paper (Pal *et al.*[4]) we have reported the *in-situ* measurements of resistivity of silver films (300 Å to 2000 Å) at room temperature. In the present paper, the resistivity (ρ) of the silver films is discussed as a function of thickness (t) of the film at 30° C by keeping the films both inside (*in-situ*) and outside (not *in-situ*) the high vacuum (Fig. 1). The resistivity of the films was found to increase drastically, especially for the thinner ones when they were removed from high vacuum. Thinner films are affected more.

In the present paper, the effect of residual atmosphere on copper films has been studied at room temperature (Fig. 2) by exposing a thin copper film (thickness 300 Å) prepared at a pressure $1 \cdot 33 \times 10^{-3}$ Newton/metre² to $1 \cdot 33$ Newton/metre², $1 \cdot 33 \times 10^{2}$ Newton/metre² and $1 \cdot 013 \times 10^{5}$ Newton/metre² (*i.e.*, atmospheric) pressure by admitting air slowly. The method of preparation of the copper films was discussed in our earlier paper (Pal *et al.*[5]). From $1 \cdot 33 \times 10^{-3}$ Newton/metre² to $1 \cdot 33$ Newton/metre² pressure, there is practically no change in the resistivity ρ of the copper film. The resistivity very slowly changes up to $1 \cdot 33$ 10^{2} Newton/metre² and finally increases abruptly as the film is exposed to atmospheric pressure. This change is very fast and the film stabilises within a few seconds. Even after annealing the previously exposed film at 120° C for about two hours at $1 \cdot 33 \times 10^{-3}$ Newton/metre², the resistivity value remained 25% higher than that obtained with the fresh sample at $1 \cdot 33 \times 10^{-3}$ Newton/metre². Murgulesch and Ionescu[6] also observed a change of 6% to 16% in the resistivity value of continuous copper films owing to adsorption of oxygen. Thus it may be concluded that one should make *in-situ* measurements on thin films in order to derive meaningful informations on size effect.

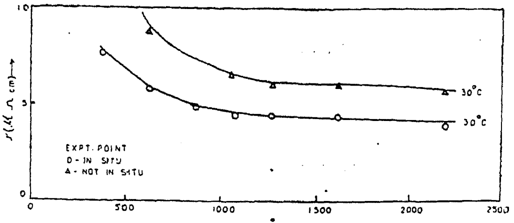

FIG. 1. Resistivity (ρ) of silver films as a function of thickness (t) at room temperature.

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* This is a part of the Ph.D. work of the author.

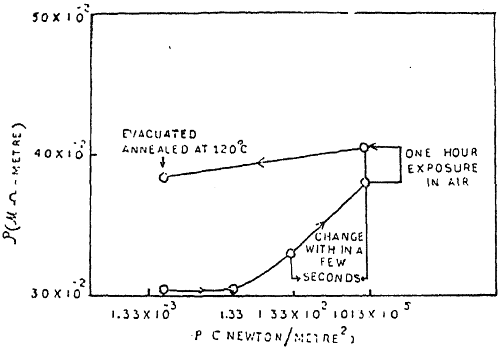

FIG. 2. The dependence of the resistivity (ρ) of a copper film (thickness = 300 Å) on the residual atmosphere at room temperature (30° C).

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1. Fuchs, K., *Proc. Cambridge Phil. Soc.*, 1938, **34**, 100.
2. Sondheimer, E. H., *Adv. Phys.*, 1952, **1**, 1.
3. Garcia, N., Kao, Y. H. and Strongin, M., *Phys. Rev.*, *B*, 1972, **5**, 2029.
4. Pal, A. K., De, U. and Sen, Paramita. *Phys. Stat. Soc.* (*a*) 1975, **27**, K. 1.
5. —, Sen, Paramita and Barua. A. K., *Thin Solid Films*, 1975, **25**, p. S. 25.
6. Murugulesch, I. G. and Ionescu, C. I., *Thin Solid Films*, 1975, **28**, p L. 35.

DIRECT SPECTROPHOTOMETRIC ESTIMATION OF CADMIUM

THE literature survey reveals that there is hardly any direct spectrophotometric methods of analysis of cadmium. The present communication reports the direct spectrophotometric estimation of cadmium with 5, 6-dibromo, 2, 3, 4-trihydroxyacetophenone (DBTHAP). The method is very simple and gives satisfactory quantitative results.

The bluish green coloured complex has a maximum absorbance at 590 nm in the pH range 9–10. The colour of the complex is stable for 20 minutes. Beer's law was varified between 20 and 90 p.p.m. of Cd(II).

The molar composition of the complex as obtained from Job's method of continuous variation was found to be 1:1. The molar extinction coefficient was found to be $1 \cdot 1625 \times 10^3$. Sandell's sensitivity is $0 \cdot 1\ \mu g/cm^2$ for $\log I_0/I = 0 \cdot 001$. The optimum concentration range for the effective determination of Cd(II), evaluated from Ringbom's plot was found to be 36 p.p.m. to 54 p.p.m.

The polarographic study of the cadmium—DBTHAP complex shows a transient existence of Cd_2^{2+}. The fact that the complex is not very stable probably explains Cd_2^{+} —DBTHAP complexation, which dissociates and cadmium is hydrolysed after some interval of time.

Interference Due to Other Ions

The effect of diverse ions was studied by adding to Cd^{2+} solution various ions ten times in excess. Ions like Hg^{2+}, Cu^{2+}, Zn^{2+}, Pd^{2+}, Ni^{2+}, Ba^{2+}, Sr^{2+}, Ti^{4+}, UO_2^{2+}, Cl^-, Br^-, I^-, CH_3COO^-, ClO_4^-, SO_4^{2-}, NO_2^{2-}, MoO_4^{2-} do not interfere. However, ions like V^{5+}, Tl^{3+}, Ca^{2+}, $Cr_2O_7^{2-}$, BiO_3^{-}, $S_2O_3^{2-}$ interfere seriously and therefore should be absent. Cations like Bi^{3+} and Pb^{2+} precipitate under the experimental conditions.

Our Sincere thanks are due to Dr. A. J. Mukhedkar and Dr. (Mrs.) V. A. Mukhedkar, Department of Chemistry, University of Poona, for their help in the preparation of the reagent.

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KINETICS OF POLYMERIZATION INITIATED BY MANGANESE(III) ACETATE

MANGANESE(III) ACETATE has been employed as one-electron oxidant for organic substrates and the mechanism of oxidation is well established[1]. Some redox systems, manganese(III) acetate-substrate, have been used to study the kinetics of polymerization of vinyl monomers[2]. But there is no report in literautre about the use of maganese(III) acetate alone as thermal and photochemical initiator of polymerization. For the first time the present study reports some aspects of manganese(III) acetate initiated polymerization of acrylamide.

Acrylamide (S.D's laboratory reagent) was recrystallised using chloroform. All the reagents used were A.R. in quality. Kinetic experiments were conducted in glass vessels (of capacity 80 ml) with an inlet and an outlet. The solutions of monomer, acid (H_2SO_4) and sodium bisulphate were deaerated for about 45 minutes. Sodium bisulphate was added to maintain constant ionic strength. Manganese(III) acetate was added after the solutions attained bath temperature. The rate of disappearance of monomer was estimated by bromometry and the rate of disappearance of manganese (III) acetate was found spectrophotometrically. The overall rates of polymerization were measured at different temperatures and the energy of activation was computed.

Manganese (III) acetate is a fairly good initiator causing a 25% conversion in 20 minutes at 40° C. The rate of polymerization of acrylamide was found to be directly proportional to the monomer concentration (*vide* Fig. 1). Another salient feature of this system is that it conforms to the ideal system where the rate of polymerization of acrylamide is proportional to half the power of the initiator (*vide* Fig. 1). The overall energy of activation is 13·9 kcal/mole suggesting that the polymerization is by free radical mechanism since for ionic polymerization the energy of activation will be very low. This suggests that manganese(III) acetate decomposes to manganese(II) acetate and accetate radical ($CH_3COO^\cdot$). The decomposition of Tl (III) acetate and initiation by acetate radical was already proposed[3]. Mutual termination of chain radicals is postulated to explain the kinetics.

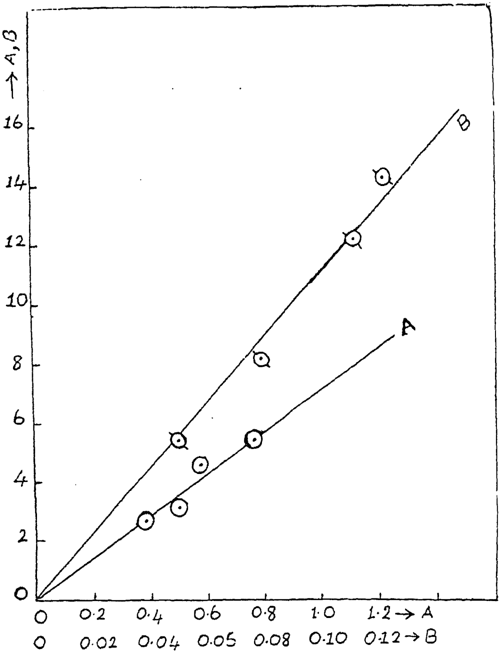

FIG. 1. Effect of monomer concentration and metal concentration on rates. T = 40° C, $t = 25$ minutes. A, $-d[M]/dt \times 10^6$ *Vs* [M], B, $-d[M]/dt \times 10^6$ *Vs* $[Mn(III)]^{1/2}$.

$$Mn\,(OAc)_3 \xrightarrow{k_d} Mn\,(OAc)_2 + AcO^\cdot$$

$$AcO^\cdot + M \xrightarrow{k_i} M^\cdot$$

$$M^\cdot + M \xrightarrow{k_p} M_1^\cdot$$

$$M_1^\cdot + M \xrightarrow{k_p} M_2^\cdot$$

. . . .

. . . .

$$M_n^\cdot + M_m^\cdot \xrightarrow{k_t} \text{Polymer.}$$

Assuming the steady state conditions and assuming k_p and k_t to remain constant and independent of chain length, the following expressions are derived :

$$-d[M]/dt = k_p/k_t^{1/2}\,k_d^{1/2}\,[M]\,[I]^{1/2}$$

and

$$-d[I]/dt = k_d\,[I]$$

A similar behaviour was also observed in the thermal polymerization of methacrylamide initiated by manganese(III) acetate[4]. The rate constants calculated were

$$k_d = 2\cdot 10 \times 10^{-4}\ \text{sec}^{-1}$$

and

$$k_p{}^2/k_t = 1\cdot 30 \times 10^{-4}\ \text{liters/mole-sec.}$$

Manganese(III) acetate also initiates vinyl polymerization photochemically to a lesser extent. Further work is in progress with various other monomers to understand the kinetics and mechanism of polymerization.

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1. Kemp, T. J. and Waters, W. A., *J. Chem. Soc.*, 1964, p. 1489.
2. Ganga Devi, N. and Mahadevan, V., *Makromol. Chem.*, 1973, **166**, 289.
3. Jayasubramanian, R., Balakrishnan, T., Kothandaraman, H. and Santappa, M., *Curr. Sci.*, 1976, **45**, 52.
4. Elayaperumal, P., Balakrishnan, T. and Santappa, M., *Die Makromol. Chem.* (in press).

CHEMICAL CONSTITUENTS OF *LORANTHUS FALCATUS* LINN. f.

IN a scheme to study and compare the chemical constituents of the host and the parasitic plants, the stems of *Loranthus falcatus* [Syn: *Dendropthoe falcatus* (Loranthaceae)][1] growing on mango trees, were collected from Ananthagiri hills for investigation. It grows mostly in deciduous forests on the host plants such as acacias and mango trees. These are parasitic shrubs, whose astringent stem bark is reported to be narcotic ; it is used locally as a cure for consumption and several other ailments[1].

Only two related species, *L. grewiniki*[2] and *L. pentandrus*[1] were examined earlier. The former contained lup-20(30)-en-3β, 7β-diol, loranthol, and the latter, quercitrin.

The alcoholic extract concentrate from the stems was fractionated successively into hexane, chloroform and methanol solubles. The hexane and chloroform extracts showed similar spots on TLC and the combined concentrate was separated on silica gel column by elution with hexane, hexane-benzene, benzene and benzene-ethylacetate.

The hexane and hexane-benzene (19:1) eluates gave rise to unidentified oils and plant alcohols. With hexane-benzene eluate (3:1) β-amyrin acetate was isolated and identified by hydrolysis to β-amyrin. In the next eluate of hexane-benzene (1:1), an apparently new triterpene, $C_{33}H_{54}O_4$, m.p. 219–220°, $[\alpha]_D + 56°$, I.R. 1735 cm^{-5} was isolated. Further elution with benzene and benzene-ethylacetate furnished β-sitosterol, stigmasterol and oleanolic acid. All the triterpenes and the plant sterols were identified with authentic samples (TLC, m.m.p. and I.R.).

The new triterpene showed no hydroxyl peak in I.R. but an ester crbonyl peak at 1735 cm^{-1}. Its NMR spectrum in $CDCl_3$ showed 7-CH_3 at δ 0·71–1–13, one acetoxymethyl at δ 2·05 (*s*) 3H, one carbomethoxyl at δ 3·63 (*s*) 3H, α-hydrogen of acetoxyl at δ 4·6 (*t*) 1 H and one olefinic proton at δ 5·33 (*m*). From the above data, the triterpene can be regarded as a methyl ester acetate of a monohydroxy triterpene carboxylic acid. On hydrolysis with 5% methanolic KOH, a hydroxy methyl ester is secured as colourless irregular plates, $C_{31}H_{50}O_3$, m.p. 196°, $[\alpha]_D + 70°$, I.R. 3450 cm^{-1} and 1735 cm^{-1}. In its NMR spectrum the acetoxyl protons were absent, but indicated one carbomethoxy at δ 3·65 (*s*) and olefinic protons at δ 5·33 (*m*) 1 H. By comparison (TLC, m.m.p. and I.R.) with authentic samples, the hydrolytic product and its parent triterpene were identified as oleanolic acid methyl ester and oleanolic acid methyl ester acetate respectively.

This appears to be first report of the occurrence of oleanolic acid as methyl ester acetate in nature. Oleanolic acid is known to occur frequently free and also as acetate[3]. In the host mango tree, *Mangifera* indica, triterpenes related to cycloartanol, namely mangiferolic acid, hydroxy mangiferonic acid[4] and a xanthone mangiferin[5], have been reported.

We wish to express our thanks to Professor M. Santappa, Director, C.L.R.I., Madras, for the NMR spectra and one of us (D.S.R.) wishes to thank C.S.I.R. for a Junior Research Fellowship.

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1. *Glossary of Indian Medicinal Plants*, C.S.I.R., India, 1956, p. 93.
2. Atta-ur-Rahman, Mohammed Ataullah Khan and Noorul Haq Khan, *Phytochemistry*, 1973, **12**, 3004.
3. Tsunematsu Takemoto, Nodoka Yahagi and Nobushige Nishimoto, *J. Pharm. Soc.*, Japan, 1955, **75**, 737.
4. Corsono, S. and Mincione, E., *Tetrahedron Letters*, 1965, **28**, 2377.
5. El Sissi, H. I. and Saleh, N. A. M., *Planta Med.*, 1965, **13**, 346.

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STUDIES ON MORPHOLINE-4 CARBODITHIOATE COMPLEX OF TELLURIUM (IV)

The fungicidal active morpholine-4-carbodithioate chelate of tellurium(IV) is stable upto 160° and after that, it decomposes rapidly upto 300°. The i.r. spectra indicate the bidentate behaviour of the ligand in the chelate. The reagent precipitates tellurium (IV) quantitatively as yellow complex in pH range 3·5–6·5. Tellurium(IV) is determined by weighing the complex as Te $(C_5H_8ONS_2)_4$ after drying at 110–120°. The reagent has also been used in the separation and determination of Te(IV) in the ternary mixture with Cu(II) and Ni(II). The interference due to Ni(II), Zn(II), Cd(II), Co(II), Fe(III), In(III) and Ga(III) ions have been avoided using ammoniacal EDTA and tartrate as masking agent.

A number of tellurium(IV) dithiocarbamates have been reported in literature[1-5]. Very little is known about quantitative estimation of Te(IV) with dithiocarbamates. Bayer and Ott[6] reported morpholine-4-carbodithioate as a possible analytical reagent. Though the reagent is not very much selective, it has been possible to determine tellurium(IV) by masking a number of metal ions using the mixture of ammoniacal EDTA and tartrate. In the present communication morpholine-4-carbodithioate is used as analytical reagent for the gravimetric determination of tellurium and separation of Te(IV), Cu(II) and Ni(II) from their ternary mixture, in addition to the fungicidal, thermogravimetric and i.r. spectral studies.

Materials and Method

The potassium salt of the reagent was prepared by mixing potassium hydroxide, morpholine and carbon disulphide in ether at 0° in a ratio 1:1:1. 1% (w/v) reagent solution was prepared in distilled water.

Potassium tellurite (Riedle) was dissolved in double distilled water to prepare the tellurium(IV) solution. The concentration was estimated by using the standard method[7].

Isolation of the Complex

A known volume of the Te(IV) solution was diluted to 150 ml and heated to 40–50°. A four-fold excess of 1% (w/v) solution of the reagent was added and its pH was adjusted between 5·0–6·5. A bright yellow precipitate gradually separated out which was digested on water bath at 50–60° for about half an hour. The precipitate was filtered in a sintered crucible (G-4), washed with distilled water repeatedly and dried at 110–120°.

The conversion factor from complex to the metal (tellurium/Te-complex) is 0·1643. The precipitation commenced at pH 3·0 and precipitation was found to be quantitative at pH range 5·0-6·5. The result of 30 estimations revealed that 2·78 mg of tellurium(IV) could be determined in a volume of 150 ml with an average error better than ± 0·5%. The complex is soluble in chloroform, carbon tetrachloride, nitrobenzene, benzene, dioxane, acetone, benzyl alcohol and ethyl acetate.

Thermal Decomposition

A known amount of the oven-dried (110–120°) sample was subjected to thermogravimetric analysis. The thermolysis curve of the complex has a horizontal portion extending upto 160° showing the stability of the complex. Between 180°–300° the complex decomposes to the extent of 35·07% and thereafter the curve again becomes horizontal.

Fungicidal Activity

The fungicidal activity of the complex (25 μg/ml in 95% alcohol) was tested on different fungi (*C. albicans, C. neoformans, T. mentagrophytes, M. canis, A. niger*) at CDRI, Lucknow and found inhibitory on *C. albican* and *C. neoformans* only.

Effect of Foreign Ions on Precipitation of Tellurium(IV)

Interference of Tl(I), Cu(II), Hg(II), Pd(II) and Pb(II) was removed by precipitating them first at pH 7·5–8·5 when Te(IV) was not precipitated. The tellurium(IV) was determined satisfactorily between pH range 5·0–6·5 in presence of Ni(II), Zn(II), Cd(II), Co(II), Fe(III), In(III) and Ga(III) using the mixture of ammoniacal EDTA and tartrate as masking agent. The ions Cl^-, Br^-, F^-, NO_3^-, $C_2O_4^{2-}$, BO_3^{3-}, Ca(II), Ba(II), Sr(II), Mg(II), Al(III) and Th(IV) did not interfere in the determination of Te(IV).

Separation and Determination of Cu(II), Ni(II) and Te(IV) in Their Ternary Mixture

When Cu(II), Ni(II) and Te(IV) were present in the solution, copper was precipitated between pH range 7·5–8·5 by masking Ni(II) using ammoniacal EDTA and tartrate mixture as masking agent. Tellurium (IV) complex was then precipitated in a slight acidic medium (pH 5·0–6·5) and finally Ni(II) was determined. The metals could be estimated with an average error of ± 0·50%.

The mixture of the three ions was taken in a beaker (500 ml) and diluted to 150 ml ; appropriate quantity of the mixture of ammoniacal EDTA and tartrate was added to mask nickel(II). The pH of the solution was adjusted in the range 7·5–8·5. 1% (w/v) reagent (KMDTC) solution was then added in excess (two fold) with constant stirring. The brown precipitate obtained was digested at 60–70° for about half an hour. After keeping for about an hour, the precipitate was filtered through a sintered crucible (G–4) and was washed repeatedly with distilled water to remove the reagent. It was then dried at 110–120° to constant weight and weighed as $Cu(C_5H_8ONS_2)_2$. The conversion factor from complex to metal (Cu/Cu-chelate) is 0·1637.

The filtrate containing Te(IV) and Ni(II) was concentrated to about 100 ml. The pH of the solution was maintained between 5·0–6·5 using acetate buffer. Tellurium(IV) complex was precipitated and determined as described above.

The filtrate of the second estimation containing Ni(II) was concentrated to about 20 ml and after cooling it, 10 ml of concentrated nitric acid (A.R.) was added, evaporated to dryness, diluted with 10 ml distilled water and again evaporated to dryness. After diluting it with 150 ml distilled water, the remaining of the nitric acid was just neutralised by 1 : 1 ammonia solution. The 1% (w/v) reagent (KMDTC) solution was then added, the yellowish green precipitate so obtained was digested at 60–70° for about half an hour. It was washed with distilled water several times, dried at 100–120° and weighed as $Ni(C_5H_8ONS_2)_2$. The conversion factor from the complex to the metal is 0·1532.

The results of estimation of Cu(II), Te(IV) and Ni(II) are given in Table I.

TABLE I

Separation and determination of Cu(II), Te(IV) and Ni(II) in their ternary mixture

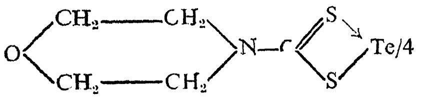
Metal Ions	Wt. of metal ions taken in mg	Wt. of complex found in mg	Weight of metal ions found in mg	% error
Cu^{2+}	(i) 12·63	77·2	12·64	+0·07
	(ii) 18·95	115·6	18·92	−0·16
	(iii) 25·26	154·2	25·24	−0·04
Te^{4+}	(i) 10·20	62·0	10·18	−0·19
	(ii) 20·40	123·8	20·34	−0·29
	(iii) 25·50	155·0	25·46	−0·15
Ni^{2+}	(i) 11·74	76·4	11·70	−0·34
	(ii) 17·61	114·6	17·55	−0·36
	(iii) 23·48	152·6	23·37	−0·46

Composition and Structure

The tellurium complex was analysed for its carbon, hydrogen and nitrogen contents to determine its composition. The composition of the complex corresponds to the formula Te $(C_5H_8ONS_2)_4$ in which tellurium to ligand ratio was found to be 1:4 (Found C, 30·98, H, 4·25 and N, 7·07, calc. C, 30·93, H, 4·15 and N, 7·20%). The conductometric titrations also confirm the above stoichiometry.

Recently crystal structure work[8] has shown that in the complex, all the four dithiocarbamate groups are bidentate and the eight sulphur atoms are bonded around the central tellurium atom in a slightly distorted dodecahedral manner. In present i.r. studies the C...N and C...S stretching frequencies in Te(IV) complex were observed at 1000 cm^{-1} for the latter and 1480 cm^{-1} for the former. In free ligand these modes were observed at 990 cm^{-1} and 1440 cm^{-1} respectively.

On the basis of above studies, the following tentative structure may be assigned to the eight co-ordinated tellurium chelate :



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1. Nikolov, G. St. Jordonov, N. and Havezov, I. J., *Inorg. Nucl. Chem.*, 1971, **33**, 105.
2. Bode, H., *Z. analyst Chem.*, 1954, **143**, 182.
3. Ryabtchikov, D. I. and Nazarenko, I. I., *Uspehi himii*, 1964, **33**, 108.
4. Gomez, J. G., Mara, U. E. and Pino, F., *Inform. Quim, Anal Madrid*, 1965, **19**, 173.
5. James, Wu, Lott, Y. L. and Droll, H. A., *Inorg. Chem.*, 1970 **9** (1), 193.
6. Bayer, V. and Ott, R. D., *Mikrochim. Ichnoanal. Acta*, 1965, p. 1130.
7. Vogel, A., *A Text Book of Quantitative Inorganic Analysis*. E.L.B.S. and Longmans, 3rd Edition, 1968, pp. 324.
8. Esperas, S. and Husebye, S., *Acta Chem. Scand.*, 1975, **29** (2), 185.
9. O'Connor, C, Gilbert, J. D. and Wilkinson, G., *J. Chem. Soc.* (A), 1969, **84**.

INDIRECT COLORIMETRIC DETERMINATION OF SOME BIVALENT METALS WITH CYANIDE AND THIOCYANATE

POTASSIUM CYANIDE or thiocyanate develops intense yellow colour with pyridine and chloramine T. The negative interference of Zn^{2+}, Hg^{2+} and Pd^{2+} in the reaction of cyanide, while that of Co^{2+}, Ni^{2+}, Hg^{2+} and Pd^{2+} in the case of thiocyanate have been utilized for their micro-determination. Using a spekol spectrocolorimeter, with absorbance at 430 nm, as little as 0·5 p.p.m. of each metal can be determined with negligible error. Stoichiometric ratios between cyanide and thiocyanate with the metal ions have been calculated.

Cyanide and thiocyanate are known to form stable complexes with most of the transition metals. Some of the methods which are used for the detection of cyanide may also be applied to the detection of thiocyanates. The method analogous to the pyrazolone was earlier[1] reported. It does not make use of the bis-pyrazolone or pyridine-benzidine reagent. The yellow colour developed with chloramine T or hypobromide is itself sufficiently intense and permits determination of cyanide or thiocyanate as low as 1–2 p.p.m. in 10 ml of the sample.

Potassium auric thiocyanate is obtained when auric chloride is added to an excess of potassium thiocyanate solution. The reaction was indirectly utilized for the colorimetric determination of gold[2] with thiocyanate. Small amounts of gold actually intensified the yellow colouration in the thiocyanate pyridine-chloramine T reaction. The metal ions like Co^{2+}, Ni^{2+}, Hg^{2+}, and Pd^{2+} interfered in the colour reaction of thiocyanate. Zn^{2+}, Hg^{2+} and Pd^{2+} interfered in the reaction of cyanide. The negative interference of these ions has been studied for the indirect determination of the metals in the present communication.

The metal salt solutions were treated with a known excess of cyanide or thiocyanate and the excess was determined colorimetrically. A calibration curve was drawn which showed a plot of the amount of the metal solution against its optical density after treatment with cyanide or thiocyanate, pyridine and chloramine T. The samples with unknown metal concentration were treated under identical conditions and the metal content was determined from the calibration curve.

The stoichiometry was established between cyanide or thiocyanate reacted with the metals. It is observed that mole of Zn^{2+}, Hg^{2+} and Pd^{2+} reacted with four moles one of cyanide. One mole of Hg^{2+} reacted with four moles of thiocyanate, but the palladium complex gave the ratio 1 : 5, this is probably due to the additional coordination and is also observed in the case of Pd(DMG)2 complex which dissolves in alkali. Stoichiometry between Co or Ni and thiocyanate could not be found because of the extreme complexing of Co^{2+} and Ni^{2+} with pyridine, thiocyanate and chloramine T.

Experimental Procedure

Varying amounts of the cation solutions (up to 5–6 p.p.m.) are treated with a known excess (about 15 p.p.m.) of cyanide or thiocyanate, 1 ml of 20% pyridine and 0·5 ml of 0·1 N (approx.) chloramine T the volume is made up to 10 ml with distilled water. The absorbance of the solution is measured on the speckol spectrocolorimeter at 430 nm. The calibration curve of absorbance against the aliquot of metal ion added is plotted.

The unknown sample of the metal is treated with the same excess of cyanide or thiocyanate under similar conditions. The absorbance was recorded at 430 nm and the amount in the sample is then deduced from the calibration curve.

Interference

Following anions and cations in a conc. of 10 fold excess do not interfere :

Anions : Cl (KCl); F^- (KF); Br^- (KBr); ClO_3^- ($KClO_3$); BrO_3^- ($KBrO_3$); IO_3^- (KIO_3); SO_4^{--} (Na_2SO_4); $S_2O_8^{--}$ ($Na_2S_2O_8$); NO_3^- ($NaNO_3$); CO_3^{--} (Na_2CO_3); CH_3COO^{--} (CH_3COONa); $C_4H_4O_6^{--}$ ($K_2C_4H_4O_5$); citrate ion (sodium salt); $C_2O_4^{2-}$ ($Na_2C_2O_4$).

Cations : Ba^{++} ($BaCl_2$); Sr ($SrAc_2$); Ca^{++} ($CaCl_2$); Li (Li_2SO_4); Mg^{++} ($MgSO_4$); Cd^{++} ($CdAc_2$); W^{+6} (sodium salt); MO^{+6} (Amm. salt).

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1. Deshmukh, G. S. and Tatwawadi, S. V., *J. Sci. and Ind. Res.*, 1960, **19B**, 195.
2. — and —, *Ibid.*, 1961, **20B**, 506.

A SIMPLE PREPARATION OF SYM.-DIBENZYLTHIOUREA

SYM.–DIBENZYLTHIOUREA (I) is a compound of considerable importance in industry as well as laboratory. Though mainly a synthetic product, it also occurs naturally in the bark of *Crataeva roxburghii*[1] (of the plant family *Capparidaceae*) and in the seeds of *Salvadora oleoides*[2] (family *Salvadoraceae*). The oil from the latter plant, whose main component is possibly I, is used as a stimulating application in painful rheumatic affections and after child birth[3]. I has also proved to be an effective drug to cause stimulation of thyroid gland function in animals[4]. Kendall[5] of Dunlop Rubber Co. Ltd. has developed I as a protectant of rubber articles against ozone-produced cracking. Similarly, Brooks and Bacon[6] of R. T. Vanderbilt Co. (U.S.A.) employed I as an accelerator in the vulcanization of rubber.

I was originally prepared by Mistry and Guha[7] in 1930 from thiourea and benzylamine. Jain[8] in 1935 prepared it by the action of perthiocyanic acid on benzylamine. A German procedure[9] involves the preparation of I by refluxing a mixture of benzylamine, carbon disulphide and toluene for 25 hours. The method reported by Lieber *et al.*[10] which involves refluxing a mixture of benzylamine and carbon diulphide has been found to be quite hazardous owing to highly exothermic and explosive nature of the reaction. I has also been prepared by the interaction of benzylamine with carbon disulphide in the presence of hydrogen peroxide and diethylamine[11]. The present communication reports a simple, smooth, and non-hazardous method for the preparation of I. The method involves the interaction of an alcoholic solution of benzylamine with carbon disulphide at 0° C in the presence of a little potassium hydroxide. The resultant product on refluxing for some time followed by cooling gives I in good crystalline shape and yield. The product may be further purified by re crystallisation from alcohol.

Procedure for the preparation of sym.–dizenzylthiourea: Benzylamine (40 ml) was dissolved in 65 ml absolute alcohol, potassium hydroxide (8 g) added, and the mixture cooled to 0–5° C. Carbon disulphide (15 ml) was then added dropwise with constant stirring. As soon as the addition of carbon disulphide was completed a semi-solid mass appeared. The whole reaction product was then refluxed for ~ 2 h on a sand bath. The clear hot solution thus obtained was then transferred to a large beaker and allowed to cool at room temperature or in the ice bath, when beautiful white and shining crystals of I were obtained. The crystalline product was filtered, washed first with water and then with ether. The product was further recrystallized from alcohol. Yield, 38 g (80%); m.p. 152°.

Besides m.p. and mixed m.p. determinations, I was also characterized by elemental analysis and infrared spectral study. Found; C, 71·00; H, 5·3; N, 10·20; S, 13·24%. Required for $C_{15}H_{16}N_2S$: C, 70·31; H, 6·25; N, 10·94; S, 12·50%. The I.R. spectrum of I gave characteristic bands at 3450 cm^{-1}, 3420 cm^{-1} (geb.), 3410 cm^{-1} and 1070 cm^{-1};

these bands have been assigned to $\nu_{(z)}$ (NH), $\nu_{(z)}$ (NH), $\nu_{(E)}$ (NH), and ν (C = S) modes respectively. The configurations of the former three bands are given by Walter and Ruess[12] as Z.Z, Z.E. and Z.E. respectively.

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1. Chakravorty, R. N., *Indian Med. Gazz.*, 1951, **86**, 152.
2. Chopra, G. R., Jain, A. C. and Seshadri, T. R., *Curr. Sci.*, 1968, **37**, 121.
3. Chopra, R. N., Nayar, S. L. and Chopra, I. C., *Glossary of Indian Medicinal Plants*, C.S.I.R., New Delhi, 1956, p., 219.
4. Friedrich, H., U., *Naturforsch.*, 1947, **2B**, 19.
5. Kendall, C. E., *British Patent*, 1962, No. 887,174.
6. Brooks, L. A. and Bacon, J. C., *U.S. Patent*, 1959, No. 2,911,394.
7. Mistry, S. M. and Guha, P. C., *J. Indian Chem. Soc.*, 1930, **7**, 793.
8. Jain, F. B., *J. Am. Chem. Soc.*, 1935, **57**, 1768.
9. Zetzsche, F. and Fredrich, A., *Chem. Ber.*, 1940, **73 B**, 1114.
10. Lieber, E., Pillai, C. N. and Hites, R. D., *Can. J. Chem.*, 1957, **35**, 832.
11. Chaturvedi, M. and Jain, A. C., *Curr. Sci.*, 1971, **40**, 156; Johar, G. S., Agarwala, U. and Rao, P. B., *Indian J. Chem.*, 1970, **8**, 759.
12. Walter, W. and Ruess, K. P., *Liebig's Ann. Chem.*, 1971, **746**, 54.

BIOCHEMICAL CHARACTERIZATION OF CASES OF MUCOPOLYSACCHARIDOSES DETECTED AT BANGALORE

MUCOPOLYSACCHARIDOSES (MPS) are a group of inherited disorders due to a faulty metabolism of mucopolysaccharides also called glycosaminoglycans (GAG). In this group there are at least six types, *viz.*, Hurler syndrome (MPS–I); Hunter syndrome (MPS–II); Sanfilippo syndrome (MPS–III); Morquio syndrome (MPS–IV); Scheie syndrome (MPS–V) and Maroteaux-Lamy syndrome (MPS–VI). All these types differ in clinical and biochemical characteristics[1]. The present paper reports observations made in nine cases of Hurler syndrome (MPS–I) detected at Bangalore.

Cases seen at the Mental Retardation (MR) Clinic of this institute formed the clinical material and a total of 1,480 cases of MR were available for this study. In all these cases fresh random urine samples were collected and subjected to various bio-chemical and chromatographic tests to detect metabolic defects. Urinary mucopolysaccharides were detected by the toluidine blue and Azure A spot tests and turbidity tests using cetylplatinyl chloride, cetyltrimethyl ammonium bromide and acid-albumin[2]. Whenever a case with excretion of mucopolysaccharides was noted, twnenty-four hour urine samples were obtained and the following confirmatory tests done[3]. Chromatography to determine the nature of MPS; ion exchange chromatography using Dowex (1 × 2; 200–400 mesh, Cl^--form) and determination of uronic acid. In addition, the plasma levels of the enzymes β-galactosidase and β-N-acetyl glucosaminidase were determined[4] using the substrates obtained from M/s. Sigma Chemicals, U.S.A.

This study led to the detection of twenty-five cases of MR who showed abnormal excretion of MPS in random urine as noted in the spot tests and also turbidity tests. It was, however, possible to obtain confirmation by repeat samples in only nine cases reported here. All nine cases showed the following salient clinical features (among others) suggestive of MPS (formerly clubbed as cases of "gargoylism"): a grotesque facies; dwarfism; thick, protruding tongue; short; stubby fingers and mental retardation. Urines from age and sex matched normal children were also simultaneously processed (to serve as controls). The results are indicated in Table I.

TABLE I

Uronic acid in 24 hour urine sample

	Age	Uronic acid mg/24 hrs.
Cases	4 ± 2·44	19·20 ± 7·21*
Controls	4 ± 2·44	5·00 ± 1·39

(* $p < 0{\cdot}01$)

In these nine cases ion-exchange chromatography showed excretion of dermatan and heparan sulfates. Also, the increased excretion of uronic acid characterised these cases as either MPS I or MPS II. Presence of corneal opacity suggested that these were cases of MPS–I. Our cases were found in both sexes, whereas MPS–II is an X-linked recessive trait; hence found only in males. The uronic acid determination showed that fractions containing dermatan sulfate had twice as much of uronic acid as in fractions containing heparan sulfate. D:H ratio is 2:1 in MPS I while it is 1:1 in MPS–II. It was also noted that the former fraction showed a 30% reduction in colour when conducted without borate in the reaction mixture.

The activity of plasma enzymes are indicated in Table II.

TABLE II

Plasma enzyme activities in Cases of Hurler's syndrome

	Beta-galactosidase*	Beta-N-Acetyl-glucosaminidase*
Patients	$0{\cdot}433 \pm 0{\cdot}120$ $(0{\cdot}01 < p < 0{\cdot}05)$	$1{\cdot}398 \pm 0{\cdot}580$ $(p < 0{\cdot}05)$
Controls	$0{\cdot}262 \pm 0{\cdot}047$	$0{\cdot}790 \pm 0{\cdot}079$

* Micromoles of substrate converted per minute per litre. The values represent mean. Range is shown in parentheses.

It was noted that there was an increase of beta-galactosidase activity, a finding which needs to be confirmed on a larger number of cases.

Metabolic disorders which are rather rare in any given population provide an opportunity to understand normal pathways and their study has thus been highly rewarding. A proper identification of the biochemical basis can also help in rational therapy and prevention of similar abnormality in a subsequent offspring.

Apart from the urine examinations to detect MPS, attempts have been made with success to diagnose specific enzyme defects in each type of MPS by leukocyte studies or by using skin fibroblasts grown in tissue cultures[4]. It has also been noted that while skin fibroblasts of MPS-I or MPS-II in tissue cultures show metachromasia and accumulation of S^{35} when tested separately, this is not seen when they are grown together[1]. This indicates that these two conditions are genetically distinct. MPS-I is an autosomal recessive trait and hence to a couple who has had one child so affected, there is a 25% chance of having a similarly affected child. However, no satisfactory therapy for MPS is as yet availale. Vitamin A, Steroids, etc., are of no value. Enzyme therapy by plasmapheresis has a short action only. Prenatal diagnosis is now possible to diagnose either syndrome. The enzyme estimations[7] can be made on the amniotic fluid cells grown in culture. Selective therapeutir abortion of affected fetuses can help reduce the number of mentally retarded and allow parents to have a normal baby.

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---

1. Dorfman, A. and Matalan, R., "The mucopolysaccoaridose" In : *Metabolic Basis of Inherited Disease*. (ed. Frederickson *et al.*) McGraw-Hill Book Company, New York, 1972.
2. Pennock, C. A., Mott, M. G. and Batstone, G. F., *Clin. Chim. Acta*, 1970, **27**, 93.
3. O'Brien, D., *Rare Inborn Errors of Metabolism in Children with Mental Retardation*, USPHS Publication, U.S.A, 1970.
4. MacBrium, M., Okada, S., Wollacott, M., Patel, V., Ho., M. W., Tappel, A. L. and O'Brein, J. S., *New Engl. Jour. Med.*, 1969, **281** (7), 338.
5. Fratantoni, J. C., *Science*, 1968, **162** (3853), 570.
6. Miluksky, A., *New Engl. Jour. Med.*, 1976, **295** (7), 377.
7. Bach, G., Friedman, R., Weissman, B. and Neufeld, E. F., *The Defect in the Hurler and Scheie Syndromes: Deficiency of L-Iduronidase.*

OCCURRENCE OF TRIPLET FOETUSES IN THE SLENDER LORIS, *LORIS TARDIGRADUS LYDEKKERIANUS* CABR.

DURING our studies on the placental and foetal development in the slender loris, *Loris tardigradus lydekkerianus*, we came across a pregnant uterus containing three foetuses. It is not uncommon in these prosimians to find twins as reported by Hill[1] and Ramaswami and Anand Kumar[2]. So far as we know, this is the first report of triplet foetuses in the slender loris. In other prosimians, such as *Cheirogaleus*[3], *Tupaia*[4], and *Microcebus*[5] usually triplets are born. Zuckermann[6] reported a single instance of triplets in *Lemur macace*.

The three foetuses reported here differ in their size and weight (Table I and Fig. 1). The largest foetus

TABLE I

Weights and lengths of the triplet foetuses

	Weight in mg.	Length in mm	
		Total length	CR length
Foetus A	1210	47	35
Foetus B	810	40	32
Foetus C	340	34	25

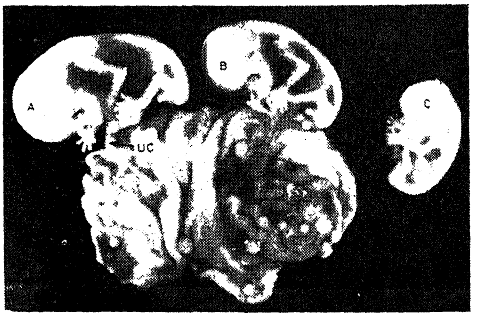

FIG. 1. Photograph of the triplet foetuses A, B and C with their placental connections. × 2 ($2\frac{3}{4}''/4\frac{2}{3}''$). UC, Umbilical cord.

(Fig. 1, A) occupied the right half of the uterus with its placenta being the most expansive. The other two foetuses (B, C) were accommodated in the left half of the uterus, the larger of the two (B) having a bigger placenta than that of the other foetus C. Sections of the ovaries of the mother revealed two corpora lutea in the left ovary and a single one in the right, indicating that the triplets are triovular. The differences in size and stage of development of the triplets may be due to delayed or deferred implantation of the left horn. This would mean that the ovulation took place simultaneously in both the ovaries and the blastocysts might have arrived later into the left horn of the uterus than in the right. The other interpretation to explain the inequality in the stages of development of the foetuses is that all the three blastocysts might have arrived simultaneously into the respective horns of the uterus but the milieu in the left horn did not permit as large a growth as in the right horn, probably due to vascular and nutritional insufficiency. However, the three foetuses do not exhibit retarded growth and the sections of the foetuses are normal when compared with those of the foetuses from single or twin pregnancies. That deferred implantation may be the more probable cause is further strenghthened by the fact that in another pregnant uterus, the right half was larger containing a bigger foetus and the left half had a smaller embryo; the ovary of this female also showed corpora lutea. If both the blastocysts had implanted simultaneously, they would have reached the same growth stage but as the left blastocyst probably arrived later, the right one had developed into a more advanced stage. The asymmetrical appearance of the uterus, however, is not of common occurrence and the reason for delayed implantation, if it occurs, is not known.

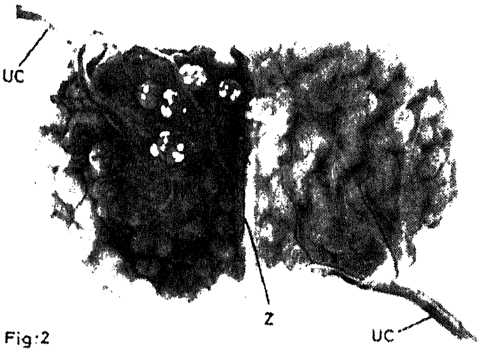

FIG. 2. Photograph of the placenta injected with Indian ink. Z. Zone of separation of the two placentae. ×2 (2⅔″/3¼″).

There are both heterosexual and isosexual twins in the slender loris and there is no free-martin effect as the placental circulation of the foetuses is distinct[2]. In the triplets reported in this paper, foetuses A and C are males and foetus B is a female as confirmed by the histological details of the foetal gonads. The maternal and foetal circulations are separate and the epitheliochorial placentae of the foetuses have no vascular anastamosis as borne out by the photograph of the placenta injected with Indian Ink (Fig. 2). Indian ink injected into the umbilical artery of one of the foetuses did not enter the placenta of the adjoining foetus as seen in Fig. 2.

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1. Hill, W. C. O., *Primates, Comparative Anatomy and Taxonomy*, Edinburgh, 1953.
2. Ramaswami, L. S. and Anand Kumar, T. C., *Acta zool. Stockh.*, 1965, 46, 257.
3. Kaudern, W., *Ark. Zool. Stockhom*, 1914, 9, 1.
4. Schultz, A. H., *Am. J. Phys. Anthropol.* N.S., 1948, 6, 1.
5. Asdell, S. A., *Patterns of Mammalian Reproduction*, Constable, London, 1964.
6. Zuckermann, S., *Proc. Zool. Soc. Lond.*, 1953, 122, 827.

EFFECTS OF TOTAL BODY SUB-LETHAL DOSE OF GAMMA IRRADIATION ON ASCORBIC ACID LEVELS IN PIGEON TISSUES

The drop of ascorbic acid in irradiated tissues has been the subject of several studies[1-4]. It has been suggested that extent of the drop in the ascorbic acid level after irradiation may be a measure of the severity of emergency situation[1]. It has also been observed that X-irradiation of ascorbic acid in aqueous solutions results in a loss of 1·7 to 2·4 μmoles of ascorbic acid after 1000 rads[2]. In biological system however, lipid peroxides formed due to irradiation are believed to inhibit the microsomal synthesis of L-ascorbic acid[6]. Many reports are available concerning effects of total body irradiation on levels of ascorbic acid in different tissues of mammals[3-5]. To our knowledge no such data are available on effects of radiation on birds. The present study deals with effects of sub-lethal dose (400 rads) of gamma-irradiation on ascorbic acid levels in tissues of pigeon.

Thirty-five pigeon (*Columba livia intermedia* Strickland) of both sexes weighing approximately 270 g were fed with a mixture of equal amounts of bajra and sorghum and tap water *ad libitum*. Animals were exposed to total body gamma-irradiation with Cobalt-60 (Theratron Junior). The radiation factors were 1·25 MeV and dose rate 99 rad/min. The animals were irradiated from the dorsal side with sub-lethal dose of 400 rads. The method of Roe[6] was used for the determination of ascorbic acid. Liver, major pectoralis muscle, kidney and spleen were removed, dissected free of fat and homogenised at 4° C with the required amount of 6% TCA and ascorbic acid was determined.

The results on the changes in the levels of ascorbic acid in the different tissues of pigeon following gamma-irradiation is shown in Table I. The distribution of ascorbic acid in control group was maximum in spleen followed by liver, kidney and muscle. The ascorbic acid was significantly decreased in muscle ($P < 0 \cdot 01$) and insignificant decrease however, was observed in liver, kidney and spleen 24 hr after irradiation.

TABLE I

Concentration of free ascorbic acid mg/100 gm fresh weight tissues of control and gamm-irradiated pigeons after 400 rads

Post-irradiation time (hours)	Liver	Muscle	Kidney	Spleen
Control	30·0 ± 1·50	10·0 ± 0·90	21·0 ± 2·10	33·0 ± 2·90
1-hour	28·0 ± 3·00	8·0 ± 0·80	18·0 ± 1·70	31·0 ± 3·00
24-hours	25·6 ± 1·30	6·0 ± 0·30 ^{c}	15·3 ± 1·50	29·3 ± 2·80
48-hours	24·3 ± 2·30	4·3 ± 0·20 ^{d}	12·3 ± 1·10 ^{c}	27·8 ± 2·70
72-hours	23·3 ± 2·10 ^{a}	1·3 ± 0·10 ^{d}	10·3 ± 1·30 ^{d}	26·0 ± 2·30

The levels of significance $a - P < 0 \cdot 05$, $c - P < 0 \cdot 01$, $d - P < 0 \cdot 01$.

After gamma-irradiation (48 hr) the significant decrease was noted in muscle ($P < 0 \cdot 001$) and kidney ($P < 0 \cdot 01$). On the other hand, after 72 hr of irradiation the ascorbic acid levels decreased significantly in muscle ($P < 0 \cdot 001$), kidney ($P < 0 \cdot 001$) and liver ($P < 0 \cdot 05$) as compared to normal non-irradiated tissues (Table I).

Ascorbic acid is known to take part in cellular respiration, protein synthesis and other metabolic functions. There have been many reports on reduced contents of ascorbic acid after irradiation in mammals. Kretzschmar and Ellis[9] have noted that the ascorbic acid of muscle and plasma are decreased immediately after irradiation and show marked decrease in adrenal ascorbic acid as well. Oster *et al.*[3] have found that mid-lethal whole body X-irradiation (710 *r*) may directly reduce the ascorbic acid content in different tissues of rat of the Long-Evans strain. Stripe *et al*[5] have also observed that the synthesis of ascorbic acid by rat liver extract was impaired after whole body X-irradiation. Anderson and Harrison[10] however, have noted that only slight destruction of ascorbic acid occurred in minced rat muscle exposed to 22,000 rads *in vitro*. In the present study even at sub-lethal dose (LD 50/30 of pigeon is 900 ± 50 rads) the ascorbic acid levels decreased significantly in muscle and kidney. Various explanations are proposed for the reduction of ascorbic acid in different tissues after irradiation. However, the following category may fit in our finding. (i) Possibly cell ascorbic acid might be destroyed by the production of oxidizing radicals of water during irradiation in the cell[11], (ii) impairment in the synthesis of ascorbic acid may occur due to the excess production of lipid peroxides after the whole body irradiation[5] and (iii) that the irradiation activates the pituitary -adrenal system which may bring down the levels of ascorbic acid as shown by Patt *et al.*[12] and Waxler *et al.*[4].

It is concluded from the present study that the sub-lethal dose (400 rads) of gamma-irradiation caused significant changes in the ascorbic acid level in the muscle and kidney of pigeon.

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1. Long, C. N. H., *Fed. Proc.*, 1947, **6**, 461.
2. Fricke, H., Hart, E. J. and Smith, H. P., *J. Chem. Phys.*, 1938, **6**, 229.

3. Oster, H. L., Kretchmar, A. L. and Bethell, F. H., *Proc. Soc. Exptl. Biol. Med.*, 1953, **84**, 470.
4. Waxler, B. C., Pancharz, R. and Thomas, S. F., *Ibrid.*, 1952, **79**, 183.
5. Stripe, R., Comporti, M. and Caprino, G., *Biochem. J.*, 1963, **86**, 232.
6. Roe, J. H., *Methods in Biochemical Analysis*, Edited by Glick, D., Interscience, New York, 1967, **1**, 115.
7. King, C. G., *J. Am. Med. Ass.*, 1950, **142**, 563.
8. Benerjee, S., *Proc. Symp. Proteins*, C.F.T.R., Mysore, India, 1961.
9. Kretzschmar, C. H. and Ellis, F., *Brit. J. Radiol.*, 1947, **20**, 94.
10. Anderson, R. S. and Harrison, B., *J. Gen. Physiol.*, 1943, **27**, 69.
11. Weise, J., *Trans. Farad Soc.*, 1947, **43**, 314.
12. Patt, H. M., Swift, M. N., Tyree, E. B. and John, E. S., *Am. J. Physiol.*, 1947, **150**, 480.

SOME NEW RECORDS OF CRUSTACEA FROM KASHMIR

HE available literature on the hydrobiology of ndian Waters pertains mostly to the plains; the ighland waters of Kashmir (over 5000 ft. a.s.l.) ave received very little attention. Only a few udies[1-5] regarding the planktonic forms of lakes nd stray ponds have been made and these too re, by and large, of a preliminary nature. There em to be no reports of similar studies on rings, some of which form chief sources of otable water and are of great tourist attraction. series of studies has been undertaken to vestigate the hydrobiology of the various water dies of Kashmir of which the present contribu- n forms a part.

During the limnological studies of Beehama ing, 24 km to the North-East of Srinagar, the lowing plankters were collected :

ROTIFERA

1. *Trichotria tetractis* Ehrenberg.
2. *Euchlanis dilatata* Ehrenberg.

CRUSTACEA

Cladocera

3. *Simocephalus elizabethae* King.
4. *Pleuroxus similis* Vaura.
5. *Chydorus sphaericus* O. F. Muller
6. *Alonella exigua* Lilljeborg.

Copepoda

7. *Macrocyclops albidus* Jurine.
8. *Eucyclops speratus* Lilljeborg.
9. *Bryocamptus* sp.

Although all the nine species are first reports from the springs of Kashmir, the following two Cladoceran species and one Copepod species are being recorded for the first time from this part of the country.

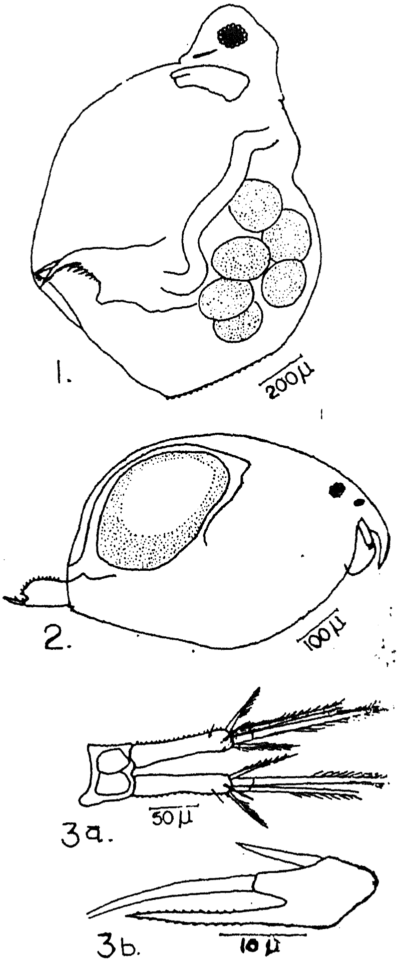
CRUSTACEA

Cladocera

Family : Daphnidae

Simocephalus elizabethae King. (Fig. 1).

A thick shell covers the large heavy body. **Valves** are large, somewhat quadrate, with rounded **angles.**



FIGS. 1–3. Fig. 1. *Simocephalus Elizabethae*, female. Fig. 2. *Pleuroxus similis*, female. Fig. 3. (*a*) *Eucyclops speratus*, caudal ramus, female, (*b*) *E. speratus*, leg 5th, female.

Small denticles are present along the postero-dorsal margin of the shell, increasing in size regularly toward the posterior extremity. A few spinules are present in the middle of the ventral margin of the valves. The head and rostrum are small and ocellus is elongated. The abdominal processes are placed far apart. Postabdomen is large, broad, truncate, posterior and emarginate and bears eight anal spines, which decrease in size toward the anal opening. Length of the adult female is 2·0 to 2·5 mm. Only a few specimens of this species were found in the samples from the spring.

Family : Chydoridae.

Pleuroxus similis Vaura. (Fig. 2)

The surface of the bivalved shell is faintly reticulated and its anterior part is sculptured by ten to twelve curved striae. Two small infero-posteal denticles are present on the shell. The head is comparatively small. Ocellus is smaller than the eye and is nearer to it than to the tip of the rostrum. The lower edge of the labrum is without any notch. Abdominal claws are of moderate size having two basal denticles. Marginal denticles are 12 to 13 in number. The adult female measures 0·5 to 0·55 mm. in length. This species was fairly present in the spring.

Copepoda

Family : Cyclopoidae

***Eucyclops** speratus* Lilljeborg. (Fig. 3 *a*, *b*).

Body is slender. The antenna consists of twelve segments, last three segments being very long and slender. The swimming legs are stout. Fifth leg consists of one distinct broad segment armed with an inner spine and two outer setae. Outer margin of the caudal ramus is provided with a longitudinal row of minute spinules. The inner terminal seta of the ramus is shorter than the ramus itself. Second and third setae are very long. Length of the adult female is 1·0 to 1·5 mm. This species was very common in the spring.

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---

1. Edmondson, W. T. and Hutchinson, G. E., *Mem. Conn. Acad. Arts Sci.*, 1934, **9**, 153.
2. Brehm, V., *Ibid.*, 1936, **10**, 283.
3. Das, S. M. *et al.*, *Kash. Sci.*, 1969, **6** (1/2), 1.
4. — *et al.*, *Ibid.*, 1970, **7** (1/2), 119.
5. Akhtar, S., *Ph.D. Thesis*, Univ. Kash., 1972 (unpublished).

EFFECT OF 'MORINDIN' A GLYCOSIDE ON THE ISOLATED HEART OF COCKROACH

GLYCOSIDES possess high physiological activity and are abundant in nature among various plants. Most of the glycosides like digoxin and digitoxin (cardiac glycosides) have therapeutic value. Glycosides from *Nerium oleander*[1] are known to possess anticancer properties and are used for heart malfunctions. As our knowledge on the effect of glycosides on insects, particularly on the heart, is fragmentary the present study has been undertaken.

The genus *Morinda* (Rubiaceae) is economically important in that the various parts of the plant are used in dyeing textiles and in medicine[2]. The glycoside "Morindin" (6–Primeveroside of Morindone, $C_{26}H_{28}O_{14}$) has been extracted and purified from the root bark of *M. tineteria* var. *tomentosa* Hook. f. (Rao and Reddy)[3]. The isolated cockroach heart technique described by Krijgsman *et al*[4] and Naidu[5] was adopted in the present studies. Test solutions were prepared by dissolving 'Morindin' in acetone (wt/vol). Nicotine (NIC), atropine (ATR) and eserine (ESR) dissolved in distilled water were use along with Morindin to study its site and mode of action. A constant dosage of 1 ml/1,000 ml physiologica saline[5] was maintained. Acetone at the dilutio employed was found to be ineffective on the hear. Each experiment was repeated at least five times an the average was taken to draw the graph. Each poin in the figure represents the number of beats observed per minute. Arrows indicate the points of additio of the test solutions.

Moindin was found to have high stimulative effect on the heart beat frequency which was sustained for a long time (Fig. 1). Atropine (a cholinegric blocker) has inhibited its action causing a sudden decline in the frequency and amplitude. Eserine (anticholinesterase) on the other hand brings about a sudden increase in the heart beat frequency (Fig. 1). This clearly indicates that acetylcholine is involved in the action morindin.

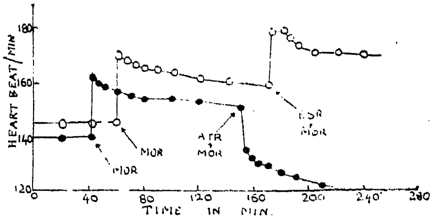

FIG. 1. Effect of morindin (5×10^{-6}), atropine (3×10^{-6}) and eserine (3×10^{-6}) on isolated cockroach heart.

After prolonged treatment with nicotine, the heart beat frequency did not increase with Morindin. When the order of treatment was reversed the effect of nicotine was unaffected (Fig. 2). Hence continuous administration of nicotine lowers the stimulating action of the cardiac ganglia and completely paralyses in about 3–4 hours (Naidu[5]). Prolonged treatment by Morindin does not paralyse the cardiac ganglia.

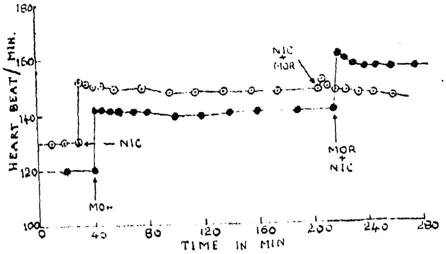

FIG. 2. Effect of nicotine (NIC, 5×10^{-6}) and morindin (MOR, 5×10^{-6}) on the isolated cockroach heart.

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1. Taylor, A., Mekenna, G. F. and Burlage, H. M., *Texas Reports on Biology and Medicine*, 1956, 14, 538.
2. Bhatnagar, S. S. *et al.*, *The Wealth of India*, A Dictionary of Raw Materials and Industrial Products, C.S.I.R., New Delhi, 1962, 6, 423.
3. Rao, P. S. and Reddy, G. C. V., *Indian Journal of Chemistry* (in press).
4. Krijgsman, B. J., Dresden, D. and Berger, N. E., *Bull. Ent. Res.*, 1950, 41, 141.
5. Naidu, M. B., *Ibid*, 1955, 46, 205.

EFFECTS OF ULTRASONICATION, STORAGE AND FREEZE-THAWING ON THE MYOFIBRILLAR-ATPase ACTIVITY OF SKELETAL MUSCLES

THE ATPase activity of the myosin has been correlated with the speed of muscle shortening[1], and this has been studied extensively in the muscles of different mammals, lower vertebrates and invertebrates. The importance of ATPase activity has also been shown with respect to the muscle structure and its regulatory mechanisms[2]. Very few data are available on myofibrillar-ATPase activity of skeletal muscles under different conditions. The present study has been, therefore, intended to obtain some useful information in this direction. Both Ca^{++} and Mg^{++}–ATPase assays have been considered, since Ca^{++} has a role to play in contractibility of muscles whereas Mg^{++} has great physiological significance in various metabolic and regulatory processes involved in the muscle study.

In the present study besides the control, experiments were carried out under four experimental conditions (Table I): (i) fresh myofibrils ultrasonicated[3] at 0° C for 2 minutes with 15 seconds intervals by sonic irradiation at 20 kc with a Bronwill Biosonik Probe delivering 120 W, (ii) fresh myofibrils were stored at 8° C and the enzyme activity assayed after 70 hours and 120 hours intervals, (iii) myofibrils frozen at –20°C for 70 hours and 120 hours, thawed to room temperature and kept in cold (2–5° C) till the assays were completed, and (iv) muscle pieces *in toto* were frozen at –20° C for 120 hours and myofibrils were prepared after thawing the muscle pieces to room temperature and keeping in cold (2–5° C) till the assays were completed.

These studies were carried out on the *Pectoralis major* muscle of common weaver bird (*Ploceus philippinus*). In all, ten animals were sacrificed, the muscle of each animal was assayed for all the above four experimental conditions and also one set for control (normal); ATPase activity was measured within 12 hours. The myofibrils were obtained basically according to the method of Perry and Grey[4]. ATPase assays were carried out at pH 7·5 and 37° C. In the Ca^{++}-ATPase assay incubation medium, 40 mM Tris-HCl, 40 mM KCl, 10 mM $CaCl_2$, 0·2 ml of myofibrils and 3 mM of ATP were used, whereas in Mg^{++}-ATPase assay the concentration of $CaCl_2$ was 0·2 mM and in addition to this 3 mM of $MgSO_4$ solution was used, the rest of the reactants were the same in the both the assays. The final volume of the reactants in both assays (Ca^{++} and Mg^{++}-activated) was 1·5 ml. The reaction was started with the addition of ATP and was stopped by the addition of 1·5 ml of 10% trichloroacetic acid. The amount of Pi liberated and protein content present were measured by the methods of Rockstein and Heiron[5] and Gornall *et al.*[6] respectively. All the values of specific-ATPase activity were expressed as μ moles of Pi liberated/mg protein/min.

It is evident from Table I that following ultrasonication, the increase in activity of Ca^{++} and Mg^{++}-ATPase assays is about 68% and 62% respectively. The increase in the activity of myofibrils after ultrasonication can be explained on the basis that sonic radiation is used to break the cell membranes and to release the cell contents; thus fragmentation may take place due to which the enzyme activity increases.

TABLE I

Effects of ultrasonication, storage and freeze-thawing on the myofibrillar-ATPase activity of skeletal muscles ATPase–activity expressed as μ moles of Pi liberated/mg protein/min.

Status of myofibril	Specific-ATPase activity			
	Ca^{++}–ATPase	P*	Mg^{++}–ATPase	P*
Control	0·302 ± 0·003		0·380 ± 0·023	
Ultrasonication	0·508 ± 0·002	< 0·001	0·615 ± 0·004	< 0·001
Storage (8° C) :				
for 70 hours	0·185 ± 0·006	< 0·001	0·227 ± 0·006	< 0·001
for 120 hours	0·166 ± 0·005	< 0·001	0·213 ± 0·004	< 0·001
Storage (– 20° C) :				
for 70 hours	0·168 ± 0·007	< 0·001	0·226 ± 0·014	< 0·001
for 120 hours	0·156 ± 0·005	< 0·001	0·205 ± 0·011	< 0·001
From frozen muscle (– 20° C) and stored for 120 hours	0·258 ± 0·005	< 0·001	0·298 ± 0·006	< 0·001

Values expressed as : means ± S.E. (standard error).

* Probability of significant difference in experimental values with respect to the controls.

Table I further shows that the values of Ca^{++} and Mg^{++}-ATPase assays during storage (8° C) and freezing (–20° C) of myofibrils decrease by 39 to 49%. However a minimum decrease in activity upto 15–20% was observed in myofibrils prepared from frozen muscle after storing for 120 hours (Table I). By and large an overall effect of various treatments was almost similar in both the Ca^{++} and Mg^{++}-activated ATPases. The decrease in the enzyme activities during storage (at 8° C and –20° C) can be explained on the basis of denaturation and also increased association from intermolecular hydrogen bonding and Van-der Waal forces. Formation of polymeric units of enzymes at low temperature could lead to decrease in specific activity[8].

On the basis of the results, it is clearly indicated (Table I) that the ATPase assay of skeletal muscle in fresh myofibrils, within 12 hours, appears ideal under normal circumstances when all facilities are available to carry out the assay. However, as revealed by the present studies, when the immediate facilities for either the preparation of myofibrils or the ATPase assay are not readily available, in cases where the work with rare animals is involved in any remote part of the world, the ATPase assay can be carried out in myofibrils prepared from frozen muscle, after storing for 120 hours at – 20° C without much loss of enzyme activity.

The fact that when the muscle is frozen at – 20° C for a period upto 120 hours, yet it shows only a 15–20% decrease in ATPase activity, further confirms the validity of the procedure of histochemically demonstrable Ca^{++} activated ATPase in muscle sections after freezing the muscle and storing at –20° C for a few days in a cryostat[9]. These studies thus reveal the pattern of differential effects of various treatments on myofibrillar-ATPase in pure isolated preparation of myofibrils.

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---

1. Barany, M., *J., Gen. Physiol.*, 1967, **50**, 197.
2. Lehman, W. and Szent-Gyorgyi, A. G., *Ibid.* 1975, **66**, 1.
3. Blanchaer, M. C. and Griffiith, T. J., *Can. J. Biochem.*, 1966, **44**, 1527.
4. Perry, S. V. and Grey, T. C., *Biochem. J.*, 1956, **64**, 184.
5. Rockstein, M. and Herron, P. W., *Anal. Chem.*, 1951, **23**, 1500.
6. Gornall, A. G., Bardawill, C. J. and David, M. M., *J. Biol. Chem.*, 1949, **177**, 751.
7. Lehninger, A. L., *Biochemistry*, Worth Publishers, Inc., New York, 1975.
8. Meryman, H. T., *Cryobiology*, Academic Press, Inc. (London) Ltd., 1966.
9. Talesara, C. L. and Narang, V., Unpublished data.

NITROGEN FIXATION UNDER RICE FIELD CONDITIONS

THE maintenance of the fertility of rice field soils for many years, without the application of fertilizers, is largely due to the microbial fixation of atmospheric nitrogen[1-2]. Among the diversified groups of microorganisms recognised as nitrogen fixers under rice field conditions, *Azotobacter*, *Beijerinckia*, *Clostridium* and blue green algae are important[3]. Soils of low-land rice fields offer a unique ecological situation with both aerobic and micro-aerophilic conditions[4]. Recently a noteworthy phenomenon has been reported by the workers at the International Rice Research Institute in the Philippines.. According to them rhizosphere microorganisms of rice plant play a very significant role in nitrogen fixation[1,5]. The observations of Dommergues *et al.*[7], through studies under tropical conditions, also confirmed this finding. The present communication examines the nitrogen fixing activity of rice crop under water-logged soils in Tamil Nadu.

A field experiment was conducted at the wetlands of Tamil Nadu Agricultural University, Coimbatore, with the rice varieties IR-20 and Bhavani raised with the recommended dose of fertilizers (60 N, 30 P and 30 K kg ha). At different stages of the crop, the rhizosphere, rhizoplane and non-rhizosphere soil samples were collected and placed in 500 ml Ehrlenmeyer flasks fitted with rubber needle puncture stoppers. Field water samples from different locations of the wetlands were also collected and taken in 20 ml rubber stoppered vials. Air in the vessels was replaced by a mixture of nitrogen and oxygen (4 : 1) and pure acetylene was injected with a Hamilton gas tight syringe to obtain *ca.* 0·05 atm. The vessels containing the rice roots were maintained at an acetylene concentration of 0·3 atm as rice roots exhibit higher nitrogenase activity only at that partial pressure of acetylene[8]. To study the contribution of the photosynthetic microorganisms towards nitrogen fixation, one set of samples was incubated under day light (*ca.* 30,000 lux) for 6 hrs.

The nitrogen fixation was studied by using the acetylene reduction technique[1]. A Perkin Elmer Model F-33 Gas Chromatograph fitted with a flame ionization detector, Porapak-N column (80 100 mesh) and a Perkin Elmer Model 56 recorder served the purpose of assaying nitrogenase. All samples were incubated at 28° C ± 1 for 24 hrs.

The data (Table I) reveal that under rice field conditions nitrogen fixation takes place under dry as well as wet conditions. The latter, however, recorded greater nitrogenase activity, because under flooded conditions. many of the heterotrophs become more active than they are in the dry soil. The data further reveal that under illuminated conditions there was more N-fixation than under darkness, indicating the role of photosynthetic nitrogen fixing microorganisms[8,9]. They warrant immediate attention for better exploitation.

TABLE I

Nitrogen fixation under rice field conditions

Samples	Incubation under	
	Darkness	Illumination
Rice field water*	10·60	17·95
Rice field soil (air dried)†	12·52	15·79
Rice field soil (water-logged)†	205·00	320·00

* nm of C_2H_4 formed/100 ml of water/24 hr.

† nm of C_2H_4 formed/kg of soil/24 hr on dry weight basis.

(Mean of ten samples collected at random)

TABLE II

Nitrogen fixation under wetland conditions in two rice varieties, IR-20 and Bhavani*

(Incubated under darkness)

Stage of the crop	Nitrogen fixation					
	Rhizosphere soil		Non-rhizosphere soil		Rhizoplane	
	Bhavani	IR–20	Bhavani	IR-20	Bhavani	IR 20
Seedling	42·00	19·00	12·00	7·00	22·80	14·00
Tillering	23·60	21·00	14·00	5·00	46·50	16·80
Boot-leaf	65·00	54·00	22·00	14·00	102·00	67·80
Flowering	95·00	64·00	18·00	8·00	86·50	71·00

(Data represent average of three estimations.)

* nm of C_2H_4 formed/100 g of soil or per g of root/24 hr.

TABLE III

Nitrogen fixation under wetland conditions in two rice varieties, IR–20 and Bhavani*
(Incubated under Illumination)

Stage of the crop	Nitrogen fixation					
	Rhizosphere soil		Non-rhizosphere soil		Rhizoplane	
	Bhavani	IR–20	Bhavani	IR–20	Bhavani	IR–20
Seedling	84·00	24·00	18·60	11·00	48·00	40·00
Tillering	46·00	52·00	22·60	16·00	53·00	65·00
Boot-leaf	136·00	76·00	38·60	18·00	72·00	190·00
Flowering	164·00	96·00	36·00	14·00	220·00	180·00

(Data represent average of three estimations.)

* nm of C_2H_4 formed/100 g of soil or per g of root/24 hr.

The two rice varieties, IR–20 and Bhavani recorded distinct differences in their nitrogen fixing capacity not only in respect of the rhizosphere but also of rhizoplane regions. In general, Bhavani registered greater N fixation activity than IR–20. At boot-leaf and flowering stages, both the varieties recorded greater activity than at any other stage. This observation adds additional evidence to our earlier finding that at boot-leaf and flowering stages of rice crop, the *Azotobacter* population was maximum in the rhizosphere[10]. In both the varieties the rhizoplane and rhizosphere samples exhibited greater nitrogenase activity than the soil farther apart. This amply illustrates that rhizosphere and rhizoplane of rice crop are much more important in nitrogen fixation than they are presumed to be. Yet another important finding of the study is that when the samples were incubated under light, better nitrogen fixation was stimulated. This could be due to (i) the contribution by photosynthetic N-fixers, (ii) through greater CO_2 fixation more of carbohydrates might have been made available to the organisms at the root zone, (iii) the possible diffusion of nitrogen into the rhizosphere region. The qualitative and quantitative changes in the microflora accompanying different growth phases of the crop need to be assessed with different rice varieties.

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---

1. Yoshida, T. and Ancajas, R. R., *Soil Biol. Biochem.*, 1973, **5**, 153.
2. Stewart, W. P. D. (Ed.) *Nitrogen Fixation* *Free-Living Microorganisms*, IBP, Cambrid University Press, Cambridge, 1975, p. 47
3. Yomaguchi, M., In : *The Fertility of Pad* *Soils and Fertilizer Application for Rice*, Fo and Fertilizer Technology Centre for As and Pacific Region, Taiwan, 1976, p. 60.
4. Yoshida, T. In : Paul, E. A. and McLaren, A. (Ed.), *Soil Biochemistry*, Marcel Dekker, Ne York, 1975, p. 83.
5. — and Ancajas, R. R., *Proc. Soil, Sci. Am* 1970, **35**, 156.
6. Dommergues, Y., Balandreau, J., Rinaudo, and Weinhard, P., *Soil Biol. Biochem.*, 197 **5**, 83.
7. Yoshida, T. and Ancajas, R. R., *Soil Sci. Pla* *Nutrn.*, 1970, **16**, 234.
8. Stewart, W. D. P., *Annu. Rev. Microbiol.*, 197 **27**, 283.
9. Takahashi, H. (Ed.), *Nitrogen Fixation an* *Nitrogen Cycle*, *JIBP Synthesis*, Universit Tokyo, 1975, **12**, 161.
10. Purushothaman, D., Oblisami, G. and Bal sundaram, C. S., *Madras agric. J.*, 1976, **6** 595.

A NEW PENTATOMID HOST FOR A PTEROMALID EGG PARASITE AND THE NATURE OF INTERSPECIFIC COMPETITION WITH OTHER HYMENOPTEROUS EGG PARASITES

REPORTS on the incidence of pteromalid parasites o the eggs of pentatomids are meagre, the only know parasites being *Pachyneuron pentatomivora* Man *Acroclisoides indicus* Ferr., *A. major* Gir. Thoug species of *Pachyneuron* are usually larval and pupal parasites of aphids, coccids, coccinellids, syrphids, chaemaemyids and anthomyids. Mani[1] reported *P. pentatomivora* parasitising the eggs of *Eurostylis punctigera* West. Bahan[2] recorded *Acroclisoides major* as egg parasite of *Tectocoris lineola* Fabr., and later *A. indicus* was described by Ferriere[3] from the eggs of an unidentified pentatomid occurring on the leaves of *Tectona grandis* L. Eulophid parasites, particularly of Tetrastichinae, are also known to parasitize pentatomid eggs, since *Tetrastichus gardeneri* Ferr., an egg parasite of an unidentified pentatomid from teak leaves[3] and *Tetrastichus* sp., attacking the eggs of *Placosternum dama* Fabr.[4], are on record. Ramamurthy[4] reported for the first time an eulophid *Anastatus colemani* Craw., and a scelionid *Asolcus* sp., parasitising the eggs of *P. dama*. The present observations report for the first time the incidence of *Acroclisoides indicus* parasitizing the eggs of *P. dama*, and an attempt has been made to study the interspecific competition between *Anastatus colemani*, *Acroclisoides indicus*, *Asolcus* sp. and *Tetrastichus* sp. for the host *P. dama*.

Eggs of P. *dama* laid in clusters on the prop roots of *Ficus bengalensis* L., were collected during the months January to May 1976 and 1977 as the reproductive potential of *P. dama* is restricted to these months, and the emerged parasites were assessed for the effect of parasitism individually.

The incidence of *Anastatus colemani*, *Acroclisoides indicus*, and *Asolcus* sp., is shown in Fig. 1. The efficiency of parasitism by *A. colemani* is high with 47·76% in 1976 and 61·69% in 1977 as compared to *A. indicus* with 4·12% and 11·88% and *Asolcus* sp. with 8·99% and 0%. Population of *Anastatus colemani* was observed to be high during March (1976) and February (1977) and the number decreases thereafter. On the other hand, the maximum incidence of *Acroclisoides indicus* was noticed in January (1976) and February (1977), while that of *Asolcus* sp., was observed to be high in February (1976). Parasitic effect of *Tetrastichus* sp., was comparatively low with the data ranging at 1·53% and 2·62% for the said period of investigation.

The efficiency of parasitism by *Anastatus colemani* appears more marked than that of *Asolcus* sp., indicating its important role as an egg parasite. The importance of *Anastatus colemani* as a promising egg parasite has also been emphasised by Mehra[6] who suggested it, as a biological control agent of *Tessaratoma javanica* Thu. The absence of competition by *Asolcus* sp., during 1977 naturally resulted in higher incidence of *Acroclisoides indicus* (11·88% as compared to 4·12% during 1976), suggesting strongly the host finding ability of *A. indicus*. This result of competition between these two species, to reach the same limited resource decided by the relative capacity of the two species to utilize the resource[5] is also visualised in the present study.

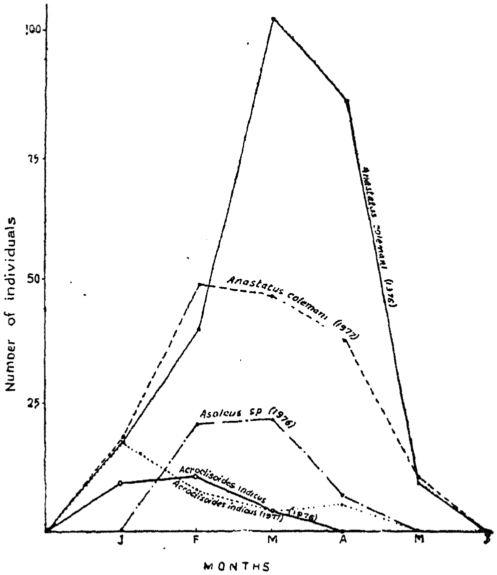

FIG. 1. The incidence of *Anastatus colemani*, *Acroclisoides indicus*, and *Asolcus* sp., parasitising the eggs of *Placosternum dama* for 1976 and 1977. (The incidence of *Tetrastichus* sp. is not shown in figure due to very low population for both the years.)

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---

1. Mani, M. S., *Catalogue of Indian Insects, Part* 3, *Chalcidoidea*, Government of India Publication, Delhi, 1935.
2. Gahan, *Philli. J. Sci.*, 1920, **17**, 345.
3. Ferriere, C. H., *Bull. Ent. Research*, 1931, **22**, 379.
4. Ramamurthy, B. N., *J. Bombay Nat. Hist. Soc.*, 1967, **63**, 766.
5. Ananthakrishnan, T. N. and Viswanathan, T. R. *General Animal Ecology*, Macmillan India, 1976.
6. Mehra, B. P. *Indian J. Entomol.*, 1966, **8** (11), 41.

A NOTE ON THE FLORAL ANATOMY OF *PRONAYA ELEGANS* HUEGL. (PITTOSPORACEAE)

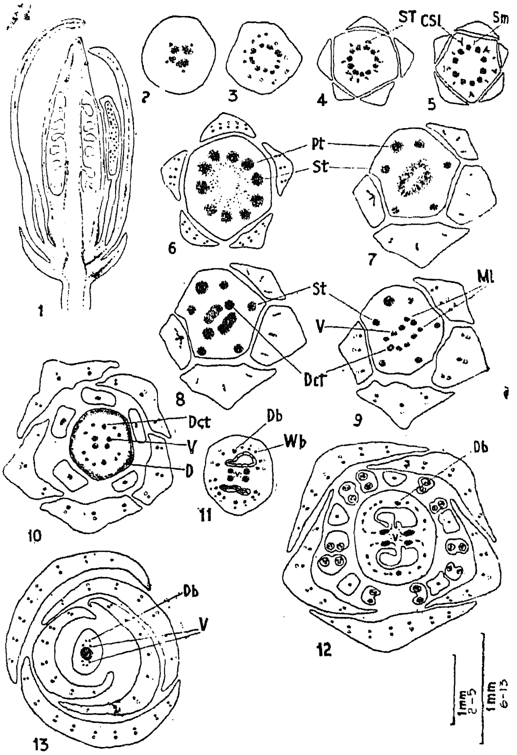
THE floral anatomy of Pittosporaceae is little known. Saunders[3] studied the floral anatomy of three taxa with a view to support her theory of carpel polymorphism. Subsequently Schaeppi[4] described the gynoecial vasculature in *Pittosporum tobira*. Recently Narayana and Radhakrishnaiah[1] have studied the floral anatomy of *Bursaria spinosa*. The present account deals with the floral anatomy of *Pronaya elegans* Huegl.

The flower is pedicellate, tetracyclic, hypogynous and pentamerous except the gynoecium (Fig. 1). The perianth is free and shows quincuncial aestivation (Figs. 4–6, 10, 12, 13). The androecium consists of five free stamens (Figs. 10, 12). The bicarpellary syncarpous ovary is bilocular throughout (Figs. 11, 12). The style is solid (Fig. 13) and the stigma is bilobed.

The pedicel shows a ring of four vascular bundles of unequal size (Fig. 2) and their number increases to ten in the thalamus (Fig. 3). The sepals are three-traced, the sepal median traces and common sepal lateral traces arising in two close alternating whorls (Fig. 4). The latter undergo radial division and demarcate the lateral traces of adjacent sepals (Fig. 5).

After the divergence of the sepal traces, the main stele shows ten prominent bundles (Fig. 6). The alterni-sepalous bundles function as petal traces and the anti-sepalous ones as staminal traces (Figs. 6–9). The traces supplying the perianth parts divide to form smaller bundles in the respective organs (Figs. 12, 13). Before functioning as petal and staminal traces they give off branches towards inside (Fig. 6), which converge to form a ring of vascular tissue (Fig. 7). From this ring of vascular tissue a pair of dorsal carpellary traces, two pairs of median lateral bundles and two pairs of ventral bundles are formed (Figs. 8, 9). The carpels are thus five-traced. The median lateral bundles, as they emerge out, divide repeatedly to form the supply to the wall of the ovary (Figs. 10, 11). Besides giving rise to the opular traces, the ventral bundles give off branches into the wall of the ovary (Fig. 12). The ventral bundles show homocarpellary pairing throughout and lie opposite the loculi (Figs. 11, 12). Judging from the position of ventral bundles, the placentation can be interpreted as axile (Puri[2]). The style is filled with transmitting tissue and vascularised by the ventral bundles and divided dorsal bundles (Fig. 13).

The disc-like basal peripheral portion of the ovary shows deep staining cells with vacuolated cytoplasm and is devoid of vascular supply (Figs. 1, 10). The vascular bundles in the floral parts are accompanied by resin canals.



FIGS. 1–13. Fig. 1. Diagrammatic longitudinal s tion showing the course of vasculature to differe floral parts. Fiys. 2–13. Serial transverse sections flower buds showing the origin and distribution traces to different floral parts. (CSI, Common sep lateral; D, Disc; Db, Dorsal bundle; Dct, Dors carpellary trace. Ml, Median lateral bundle; Pt, Pe trace; ST, Sepal trace. St, Staminal trace; V, Vent bundle; Wb, Wall bundle.)

We are thankful to Dr. J. S. Beard for the mater and Prof. Swami for facilities.

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---

1. Narayana, L. L. and Radhakrishnaiah, I "Floral anatomy of Pittosporaceae—I," *Jou Jap. Bot.*, 1976, **51** (9), 278.
2. Puri, V., "Placentation in angiosperms," *Bot. R* 1952, **18**, 603.
3. Saunders, E. R., *Floral Morphology*, Vol. W. Heffer & Sons Ltd., Cambridge, 1939.
4. Schaeppi, H., "Zur Gestaltung des Gynoeceu von *Pittosporum tobira*," *Ber. Schweiz. I Ges.*, 1971, **81**, 40.

ANTIGENICITY AND CROSS-REACTIVITY IN *EPIDERMOPHYTON FLOCCOSUM*

IMMUNITY in dermatophytoses has been a debated subject[1], although circulating antibodies to dermatophytes in humans and experimental animals have been demonstrated earlier[2]. Water soluble and neutral polysaccharides from species of *Trichophyton* and *Microsporum* have been shown to be antigenic in rabbits[3]. Information on the antigenic potentialities of *Epidermophyton floccosum* on the other hand is meagre and poorly understood. The present study reports the antigenic structure of several clinical isolates of *E. floccosum* and the cross-reactivity among themselves and between dermatophytes.

The antigen for immunizing rabbits was prepared from a 15-day old shake culture of the fungus grown at 30° C in Sabouraud's glucose broth. Vacuum dried mycelial powder suspended in saline (100 mg/ml) was autocalved at 15 lb for 20 minutes. The suspension was then homogenized and mixed with an equal volume of complete Freund's adjuvant. Soluble antigens for *in vitro* tests were prepared by grinding mycelial powder with glass powder followed by extraction with borate-saline buffer (pH 7·2). The antigen extract contained 6 mg protein/ml.

Each of four rabbits (weighing 1·5 to 2·0 kg) in a group was injected with 0·2 ml of antigen-adjuvant mixture (0·6 mg protein) subcutaneously on days 1 and 8. The dose was doubled and trebled on 15th and 22nd days, respectively. One booster dose of soluble antigen (1·2 mg protein in 0·2 ml) was injected through the ear vein on the 29th day. During the entire immunization each rabbit received antigen equal to 5·4 mg protein. The blood was collected through the ear vein, one week after the last injection and the serum was separated.

The antibodies in the serum were detected by ring (qualitative precipitin) test, haemagglutination and complement-fixation tests, and by gel diffusion and immunoelectrophoretic methods as described by Campbell *et al.*[4]. The banding pattern of antigen-antibody interaction in gel diffusion plates and recording the results are according to the method of Kalyanasundaram *et al.*[5].

The serum from the rabbits immunized with *E. floccosum* showed the presence of antibodies. The titre values by ring test, haemagglutination and complement-fixation tests were 1:80, 1:256 and 1:32, respectively. The titre values remained unchanged after second and third booster doses of antigen. Gel diffusion and Immunoelectrophoretic tests revealed the presence of three distinct antigenic components (numbered 1 to 3) in *E. floccosum*. The soluble antigen used in *in vitro* testing for the presence of antibodies, contained glycoprotein comprising of 6% protein and 35–40% polysaccharides.

The results revealed that all dermatophytes tested with the exception of *Trichophyton violaceum* HM 354, have cross-reacted with antiserum raised against *E. floccosum* HM 308*a* (Table I). The study further points out the commonness of one antigenic component (band 3) for all the fungi. Two isolates of *E. floccosum* (HM 116*a* and HM 269) are in complete homology with HM 308*a*, serologically, whereas the homology in five other cases has been only partial being represented by two of the three antigenic components only. In five other isolates of *E. floccosum* the cross-reactivity is indicated by only one antigen (band 3) which is also present in species of *Trichophyton* and *Microsporum*. Thus the cross-reactivity varied greatly among isolates of *E. floccosum*. *T. mentagrophytes*, *T. rubrum*, *M. gypseum* and *M. canis* possessed only one antigen common with *E. floccosum*. These isolates were cultured from clinical lesions of types tinea cruris, t. circinata, t. corporis and onychomycoses. But no correlation was observed between the antigenic composition of dermatophytes and clinical lesions they produced. The antigenic differences among isolates of a single species must be borne in mind while preparing test vaccines.

TABLE I

Antigenic dissimilarities among isolates of Epidermophyton floccosum *and between* E. floccosum *and other dermatophytes*

Antigen isolated from		Antiserum against	Precipitin bands observed
Species	Isolates		
E. floccosum	HM 308 *a*, HM 116 *a*, 269	*E. floccosum* HM 308 *a*	1, 2, 3
do.		do.	1, 2, 3
do.	HM 14, 219	do.	1, 3
do.	HM 145, 167, 358	do.	2, 3
do.	HM 78, 264, 289, 290 *a*, 314	do.	3
Trichophyton mentagrophytes	HM 115	do.	3
T. rubrum	HM 123	do.	3
T. violaceum	HM 354	do.	0
*Microsporum gypseum**		do.	3
M. canis	HM 382	do.	3

* A strain obtained from London School of Hygiene and Tropical Medicine, London.

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1. Kligman, A. M. and DeLamater, E. D., *Ann. Rev. Microbiol.*, 1950, **4**, 283.
2. Grappel, S. F., Blank, F. and Bishop, C. T., *Dermatologia*, 1971, **143**, 271.
3. —, — and —, *Bacteriol. Rev.*, 1974, **38**, 222.
4. Campbell, D. H., Garvey, J. S., Cramer, N. E. and Dussdorf, D. H. In *Methods in Immunology*, Publ. W. J. Benjamin, Inc., N.Y., 1970, Vol. 2.
5. Kalyanasudaram, R., Desai, V. B. and Sirsi, M., *Phytopath. Z.*, 1967, **59**, 39.

BERLESIELLA (FAM. PLEOSPORACEAE) A NEW MYCOPARASITE FROM INDIA

DURING the mycological collection tour of Goa in 1971, an unusual fungus was observed, in the form of whitish lesions overgrowing the stromata (discothecia) of another fungus, *viz.*, *Rhytodhysteron* (= *Tryblidiella*) which on microscopic examination was identified as a species of *Berlesiella* Sacc. (Fam. Pleosporaceae), an ascomycete which was found to be hitherto undescribed from India[2]. The taxonomy of this fungus is in dispute. Chenantais[1], in his classic paper, discussed this aspect of speciation in this genus at length and accepted only two valid species, *i.e.*, *B. setosa* Wint. and *B. nigerrima* (Blox.) Sacc.

The writers' collection was, therefore, studied in detail, which on comparison with the two known species of *Berlesiella*, was found to differ significantly in morphology and dimensions of various fruiting structures (*vide* Table I) and as such described here as new to science.

Berlesiella castle-rockii sp. nov. (Fig. 1, A–E)

Pseudothecia pulvinata, hemispherica vel oblonga, carbonacea, discreta, solitaria, ostiolata, magnit. 68–104 × 108–144 μm; asci octosporae clavatae, bitunicati, magnit. 44–52 × 6–10 μm; ascospora fusoidia vel oblonga muriformia, olivacea, magnit. 12–16 × 4–8 μm.

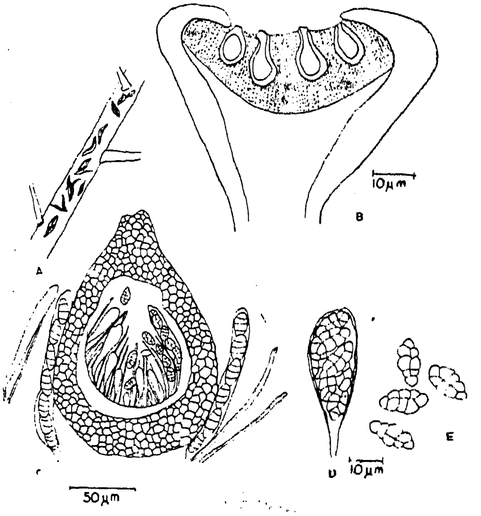

FIG. 1. (A–E): *Berlesiella castle-rockii* sp. nov. A, Habit. B, V.S. of *Rhytidhysteron* showing relation of mycoparasite; C. Pseudothecium of *Berlesiella* in section; D, Ascus; E, Ascospores.

Pseudothecia pulvinate, hemispherical to oblong, carbonaceous, discrete; solitary, more or less beak-like structures with an ostiole, measure 60–104 × 108–144 μm; asci octosporous, clavate, bitunicate, measure 44–52 × 6–10 μm. ascospores fusoid to oblong, muriform, greenish, measure 12–16 × 4–8 μm. *Matrix*: Mycoparasite on the discothecia of *Rhytidhisteron rufula* Spreng, growing on stems of *Eleagnus conferta* Roxb. (Fam. Eleagnaceae) Legit. V. G. Rao at Castle Rock, 1971, No. AMH 2074 (Holotype).

Grateful thanks are due to Prof. M. N. Kamat for his keen interest and encouragement, to the Director, M.A.C.S. Research Institute, Poona 4, for facilities

TABLE I

Comparison between species of Berlesiella

Species	Stroma	Asci	Ascospores	References
B. nigerrima (Blox.) Sacc. (type spp.)	Upto 1 mm across	80 × 12 μm	12–17 × 5–6 μm	Chenantais[1]
B. setosa Wint.	..	120 × 15 μm	24–26 × 8–10 μm	Chenantais[1],
B. castle-rockii (under study)	108–144 × 68–104 mμ	44 – 52 × 6–10 μm	12–16 × 4–8 μm	Authors

the Ministry of Education, Government of for the award of S.R.T. to one of the authors W. S.).

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Chenantais, J. E., *Bull. de la Soc. Mycol. de France*, 1921, 37, 64.

Mukherji, K. G. and Juneja, R. G., *Fungi of India*, Emkay Publ., Delhi, 1975, p. 224.

ISOMERISM IN SPATHES OF *XANTHOSOMA VIOLACEUM* SCHOTT.

an earlier paper Reddy and Bahadur[5] have ted left- and right-handedness with regard to the of spathe either to the left or right and also ied their frequency in four genera of Araceae. ness in *Xanthosoma violaceum* was also reported. In this note we report observations manner in which the spathes show contortion in a spadix pair, produced in the axil of the foliage in the Botanic Garden, Kakatiya University, Warangal, have been diagrammatically represented in Fig. 2 (A to D). In a given spadix the fleshy spike is subtented by a white to violet spathe which contorts either to the left or to the right. Generally one pair or spadices is produced in the axil of the successive leaves. In a pair the contortion of the spathes varies and four well defined combinations have been observed. There are two involucres (I_1 and I_2) produced in the axil of the leaf (LF). The one on the left (I_1) is linear and holds a spadix (S_2), whereas the other (I_2) is keeled and furrowed. The second spadix (S_1) arises in the furrowed involucre on to the left and flowers earlier than S_2. Since the spadices are produced in pairs, there are two pure combination (Right–Right or R–R) and (Left–Left or L–L) and the remaining two may be called mixed combination, viz., (R–L) and (L–R). It is interesting to note that the four spadices pairs are mirror images and hence bioisomers. Correlated with this bioisomery is the mathematical isomery *i.e.*, the left- and right-handed bioisomers occur in equal ratio (Reddy and Bahadur[5]). It is strange to note that the pure combinations were much more frequent than the mixed ones. Although the numerical data are meagre, it would be interesting to investigate this phenomenon. Occasionally S_1 was replaced by a foliage leaf but the

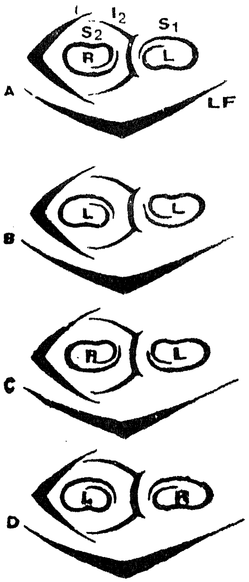
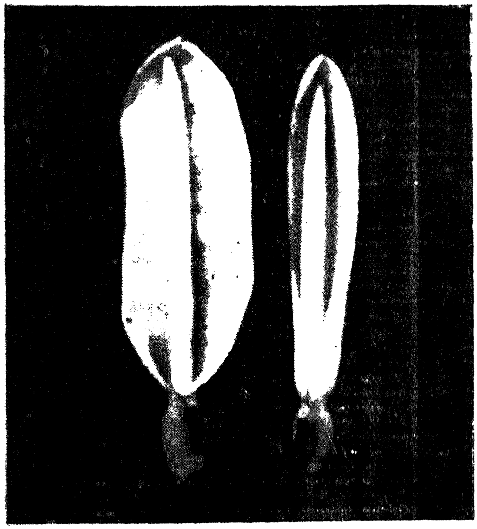


FIG. 2 (A to D). Diagrammatic representation of spadices and their relative position with the involucres in the leaf axil.



1. Photograph showing right and left handed of *Xanthosoma violaceum*. Note the contortion of spathes as indicated by the black paper strip

Fig. 1 right- and left-handed spadices of *violaceum* have been shown. The various spadix combinations observed during the last four years contortion was found to be once again identical forming mirror image pattern. The mirror image pattern thus described in *Xanthosoma* is comparable to the one described in various Araceae by Reddy and Bahadur[5] and even with the cyathia of *Euphorbia milii,* where there are only two bracts which contort in clockwise or counter-clockwise pattern and form various clustering patterns (Bahadur and Reddy[2]).

Whether the bioisomerism of spathes in *Xanthosoma* is genetical or chemically controlled is presently not known. The hypothesis that biological handedness variously termed bioisomerism or isomerism (Bahadur and Venkateshwarlu[3]) is due to stereoisomerism of hormone molecule appears to be attractive as suggested by Bahadur and Reddy[2]. Alternately, Bahadur and Venkateshwarlu[3] have also suggested that optically active, *i.e.*, levo and dextro compounds determine handedness in biological objects. The molecular chirality is thus expressed in biological chirality. Onslow observed that, "The plant form is an expresion of its chemical constitution" (Arber[1]). Recently, however, Kasinov[4] has shown that the left and right handedness in fronds of *Lemna gibba* (Lemnaceae) is inherited, although the fronds transmit asymmetry by vegetative reproduction only.

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1. Arber, A., *Monocotyledons.*, Hafner, N. Y., 1961, (Reprint).
2. Bahadur, B., and N. P. Reddy, *New Phytol.*, 1975, **75**, 131.
3. — and Venkateshwarlu, T., *J. Indian Bot. Soc.*, 1976, **55**, 30.
4. Kasinov, V. B., *Genetika*, 1969, 5, 22.
5. Reddy, N. P. and Bahadur, B., *J. Bombay Nat., Hist. Soc.*, 1976, **73**, (2).

BACTERIAL LEAF SPOT OF BANYAN—A NEW DISEASE IN INDIA

A SEVERE leaf spot disease was observed on trees of 'Banyan' (*Ficus bengalensis* L.) during winter of 1973 in Aurangabad. In nature, the disease was manifested as foliar spotting and necrosis. The small spots, later coalesced to form irregular patches, some remaining isolated and dark in colour. Each spot had a greyish centre surrounded by dark coloured border. As the disease advanced the affected leaves dropped down. Young leaves were more diseased than the old ones.

In repeated isolations, a yellow pigmented bacterium, growing as circular, flat, smooth, glistening and butyrous colonies with entire margins on NA, was obtained.

The phytopathogenic organism was gram-negative, rod-shaped, $0 \cdot 5-1 \cdot 2 \times 0 \cdot 2\ \mu$ in size, single, capsulated, non-sporing and non-acid fast. The biochemical and physiological characteristics of the bacterium were, strictly aerobic, gellatin liquified; starch not hydrolysed; ammonia, hydrogen sulphide and catalase produced but not indole; nitrate reduced; Voges-Proskauer and methyl red tests positive; milk peptonized without coagulation; litmus reduced; acid produced without gas from glucose, lactose and sucrose; amino acids not decarboxylated.

The cultural, morphological and physiological properties of the phytopathogenic organism indicated that the bacterium belongs to the genus *Xanthomonas.*

The xanthomonad was found to be specific to *F. bengalensis* as it failed to infect other 6 hosts when tested. The incitant of the leaf spot of 'Banyan' is, therefore, identified as a new species and the binomial *Xanthomonas ficae* sp. nov. is proposed.

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AWARD OF RESEARCH DEGREES

Utkal University, Bhubaneswar, has awarded the Ph.D. degree in Chemistry to Shri Sashi Bhusan Mohanty; Ph.D. degree in Zoology to Shri Hari Sadhan Maity.

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